OBSERVATIONS ON CHICKEN TUMORS CAUSED BY FILTERABLE AGENTS.

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A TRANSMISSIBLE AVIAN NEOPLASM (SARCOMA OF THE COMMON FOWL)

By PEYTON ROUS

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A TRANSMISSIBLE AVIAN NEOPLASM.[1] (SARCOMA OF THE COMMON FOWL.)

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PLATES LXVI–LXVIII.

Among the many recent observations on transmissible neoplasms are several which may have greatly enlarged our knowledge of tumor behavior and certainly, for the present, have somewhat confused it. The tumors of the lower animals first studied experimentally—those of the rat and mouse—were found to conduct themselves much as do human neoplasms; and results with them rather strengthened than changed our conception of tumor-characters. But there have since been discovered a number of transmissible new growths of unusual behavior, among them a sarcoma of the dog, transmissible at coitus (Sticker, Ewing), an endemic carcinoma of fishes (Plehn, Pick, Gaylord), and a new growth of hares (von Dungern and Coca), transplantable to animals of another species. All of these in their conduct differ more or less markedly from the classical neoplasms, and whether they are to be accepted as genuine tumors is still doubtful. On the other hand, it is possible that our conception of tumor-behavior, based as it is on observations among few species, has been too narrow.

At this time then the discovery and study of transmissible tumors in new species or classes of animals has an exceptional value. And it is for such reason that a sarcoma of the chicken—the first avian tumor which has proved transplantable to other individuals—will here be dealt with in some detail.

New growths are not rare among birds, and those of the common fowl have had attention from several investigators. Fibromas, myomas, lymphomas, carcinomas and sarcomas, some of them with

[1] Received for publication June 13, 1910.

metastases, have been described. Ehrenreich,[2] and Tyzzer and Ordway[3] have made attempts at transplantation, but without success, if one except an auto-transplantation of a lymphoma accomplished by the latter authors. Ellerman and Bang[4] have shown chicken leukemia to be transmissible, and in some of their animals aleukemic lymphomata resulted from inoculation. But they have also shown, as have Hirschfeld and Jacoby,[5] that the disease is dependent on a filterable virus.

The tumor here reported was found in a barred Plymouth Rock hen of light color and pure blood. It had existed for some two months before the fowl was brought to the laboratory, becoming noticeable when the host was about fifteen months old. The sarcoma described by Ehrenreich and the myxosarcoma of Tyzzer both occurred in adult fowls.

In this hen there was present, projecting sharply from the right breast, a large, irregularly globular mass. It had developed slowly, and without apparent involvement of the health of the host. Operation was done under ether and nearly all of the growth removed. When sliced it was found to have undergone a widespread coagulation-necrosis at the center, but there was a rim of translucent, rather friable, yellowish-pink tissue of glistening, finely striated surface. Macroscopically, the growth suggested a sarcoma. Bits of it were at once inoculated by means of a trocar into the other breast and peritoneal cavity of the host. Like inoculations were also made into two hens from the same setting of eggs. Thirty-five days later the original host was dead of intraperitoneal growths, and in the breast of one of the other fowls, a large nodule had developed. A summary of the protocols follows.

Oct. 1, 1909. *Operation on Original Tumor.*—The fowl bearing the growth is a strong, young hen. The mass is situated on the right breast, in the subcutaneous tissue, and is somewhat movable. It is irregularly spherical in shape, firm, smooth, well-defined, and projects sharply from the breast contour. It

[2] M. Ehrenreich and L. Michaelis, *Zeit. f. Krebsforsch.*, 1906, iv, 586; M. Ehrenreich, *Med. Klin.*, 1907, iii, 614.

[3] E. E. Tyzzer and T. Ordway, *Jour. of Med. Research*, 1909, xxi, 459.

[4] V. Ellerman and O. Bang, *Cent. f. Bakt., Orig.*, 1908, xlvi, 595; *Zeit. f. Hygiene u. Infektionskrank.*, 1909, lxiii, 231.

[5] H. Hirschfeld and M. Jacoby, *Zeit f. klin. Med.*, 1909–10, lxix, 107.

measures 4.8 by 4.1 by 4.1 centimeters. At its outer pole the skin is attached and thinned, with several slight ulcerations over which are dry, dark-red crusts. Elsewhere, the skin is uninvolved, though the subcutaneous fat is thinned.

Under ether most of the mass was to-day excised, a piece 1 by 3 by 0.5 centimeters being left *in situ*. The growth shelled out easily from the surrounding tissue, and was enclosed in a capsule well supplied with blood-vessels.

The part removed is found on section to consist of a rim of translucent, rather friable tissue about a center that has undergone coagulation necrosis and is firm, yellow and opaque. The rim-tissue is glistening, yellowish-pink, finely striated. No debris comes away from it on scraping, and its surface remains smooth. The region of coagulation-necrosis is serpiginous in outline and forms much the larger part of the mass.

Implantation was made by means of a large trocar into the muscles of the left breast of the same fowl and also into the peritoneal cavity. Bits of the tumor-rim approximating 0.03 cubic centimeter were thrust into each site. Similar implantations were carried out on two young hens of the same brood. No cultures were taken at this time.

Nov. 5, 1909. *Autopsy of the Tumor Fowl, Which Died Yesterday.*—The fragment of the original growth is no longer to be found, but in the muscle of the left breast is an ovoid mass 1.5 by 1.5 by 2.2 centimeters, with similar necrotic center and translucent marginal zone. It has no definite capsule and is not sharply separated from the muscle about it. The peritoneal cavity contains about 20 cubic centimeters of a thin, straw-colored fluid. Attached to the lower margin of the liver, to the oblique membrane, and to the parietal peritoneum are many firm, pale yellow, ovoid or globular nodules, the largest about 1 centimeter in diameter. On section these resemble the nodule in the left breast, except that in them the necrosis is irregularly distributed. At the pelvic region, where several of the masses have coalesced, softening and necrosis are extensive. Smears from fragments taken here show no tubercle bacilli; but with aqueous methylene blue a large rod-shaped bacillus, presumably a post-mortem invader, is demonstrable. No growths are present in the other organs.

Nov. 5, 1909. In the muscle of the left breast of one of the young hens inoculated on October 1 is a firm mass measuring 2.5 by 3.5 centimeters. (The remainder of this protocol is given further on.)

Microscopic preparations of the original growth, and of the nodules developing elsewhere in the host on implantation, have shown it to be a spindle-celled sarcoma. The growths from all latter transplantations have yielded similar pictures, so the histology of the tumor will now be taken up.

In a typical section one observes loose bundles of spindle-cells coursing in every direction, and separated from the lesser blood-vessels only by endothelium (Plate LXVII, Figs. 3 and 4). Where such a bundle is cut transversely, the appearance is that of a group of round cells of varying sizes. With Mallory's phosphotungstic

acid stain intercellular fibrils are demonstrable, though they are rare in the more cellular portions of the growth. The spindle cells, while in general large, vary much in size and shape; some are short and plump, some continued into long, slender processes. The nucleus is, as a rule, large and vesicular, with a loose network and several coarse masses of chromatin. Occasionally, it is rod-shaped, and not seldom pyknotic. To the more slender cells, it gives a bulged outline at that region where it is located. Mitosis is fairly frequent and cells with two to five or six nuclei are not rare. These small giant-cells give to the growth here and there a somewhat polymorphous appearance. They are especially to be found where necrosis is beginning. The widespread necrosis seems in general dependent on insufficient vascularization, although hemorrhage from the thin-walled vessels is also occasionally responsible. In some of the later growths a myxomatous degeneration has separated the cells, and in at least one there has followed a liquefaction with formation of a cyst.

The original tumor was better encapsulated than those resulting on transplantation. Indeed, it gave clinically the picture of a benign growth until after its dissemination at operation. The apparent liberation from restraints that took place then is a phenomenon that has been noted by others[6] for the neoplasms of rats and mice. Infiltration has been the rule since. Search shows that it was also present to some extent in the original growth before operation, despite the considerable encapsulation. The tumor cells had pressed through this capsule here and there and invaded the muscle. The muscle fibers in their breaking down furnished on transverse section some remarkable pictures of pseudo-giant-cells (Plate LXVIII, Fig. 5).

Following the growth's successful transplantation, an attempt was made to propagate it further. At this writing, it is in its fourth generation. The results bring out in an interesting way the importance of blood-relationship to the transmission. The tumor has never thriven except in the intimately related fowls of the pure-blood stock in which it was first noticed. The members of this stock were few and their relationship to one another can be stated with considerable accuracy.

[6] L. Loeb, *Jour. of Med. Research*, 1901, vi, 28.

The tumor was found in one of six hens of pure blood from the same setting, and these, with a single cock of pure blood from another source, formed the parent birds of the stock. From them a single generation of chickens had been raised when the tumor was noted. For the first transplantation two of the parent hens (of the same setting as the tumor fowl) were used, but in the later ones the younger generation of chickens was employed. These, which numbered in all only between twelve and fifteen, had at least one parent in common, some of them two, and some may have been the offspring of the tumor hen, or of that in which the neoplasm grew on its first transplantation. It would be interesting to know the exact relationship between these two hens and the young fowls that proved susceptible, but that cannot be ascertained because the eggs were mixed indiscriminately for setting. Yet it is evident enough that the relationship between all of the fowls of the special stock was a very close one.

Out of twelve of this stock to which it has been transplanted, the tumor has grown in three, and they have been the hosts for the tumor generations thus far accomplished.[7] In sixteen market-bought Plymouth Rocks, superficially like the tumor stock but presumably of impure blood, no growth has been obtained; nor has it occurred in five chickens of mixed breed. In two of three market-bought Plymouth Rocks, which were less than three months old, a transient growth, followed by retrogression, was noted (Chart 1). In two pigeons and in two guinea pigs the results have been negative.

The transplantation appears to succeed better in young fowls, judging from its partial success in the young, market-bought Plymouth Rocks and its complete failure in adults of the same sort. At present, the tumor, while still growing in hosts of the special stock, is growing slowly; slow growth may be attributable to the fact that no young fowls are available, for all of the chickens of the second generation of this stock are now adult.

Only two fowls thus far have died as a result of the tumor, which attains a large size before the general health is much affected.

[7] Since this was written transplantation to similar fowls of pure blood from another source has proven successful.

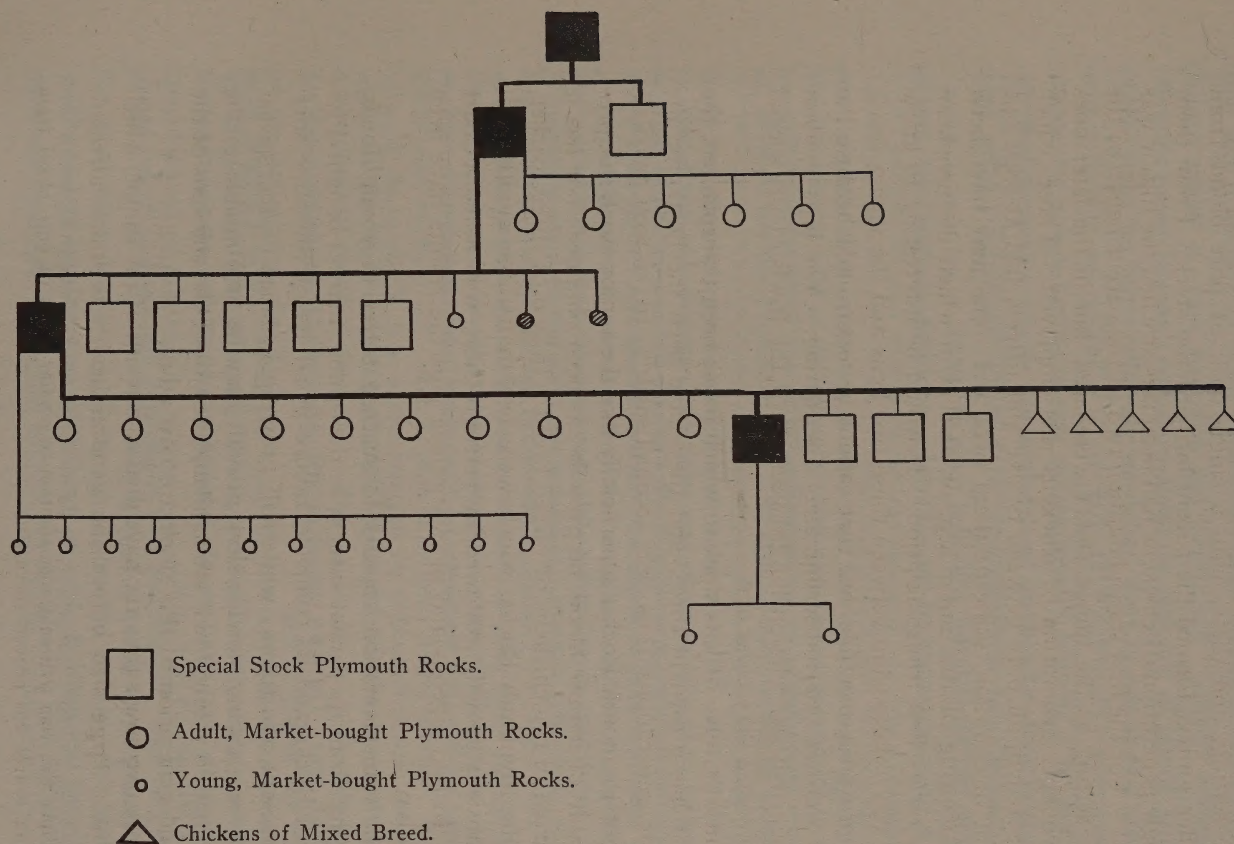

CHART I. Chart showing the course of transmission of the chicken sarcoma. The blackened figures represent individuals in which the tumor grew; the cross-barred ones those in which a nodule appeared but retrogressed.

(Plate LXVI, Figs. 1 and 2). Thorough bacteriological examinations of the neoplasm have twice been made at operation, but with negative results. Metastasis has occurred once, a tumor nodule being found in the left ventricle of a hen (second generation B) from which the growth had been partially removed some weeks previously (Plate LXVIII, Fig. 6). Whether the operative procedure or a natural dissemination was responsible for this metastasis cannot, of course, be determined. Many intraperitoneal inoculations, and reinoculations of negative individuals have been made, but growth has followed none of these.

A brief summary of the remaining protocols to date will now be given.

First Generation A.—Nov. 8, 1909. One of the 2 young hens inoculated with bits of the original growth was observed 3 days ago to have in the left breast a mass measuring 2.5 by 3.5 centimeters. This was operated upon today and found to be a tumor-nodule. Bits of it were transferred with the trocar to the fowls of *second generation A.*

Dec. 12, 1909. Following operation on the nodule it grew rapidly and to-day measured 5.3 by 4 by 3.5 centimeters, when it was again cut into for the sake of inoculation to *second generation B.* Hemorrhage was so profuse, and the mass was found to extend so deeply into the breast tissue that the fowl was killed. At autopsy, in addition to the mass on the breast, there was present a discrete, firm, translucent, grayish-pink nodule, 0.4 centimeter in diameter, in the substance of the left ventricle. Microscopically, this proved to be a metastasis. The other organs were normal. Fragments of the large growth were placed in bouillon and litmus milk, on sheep-serum agar, plain agar, and Loeffler's blood-serum. Also cultures were taken on gelatine, glucose bouillon, potato, the peptone medium, and glucose agar. These were under observation for several weeks. Bacterial growth occurred in none of them. The Loeffler's blood serum was quite actively digested by the fragment resting on it. Direct smears from the fresh tissue were examined for bacteria, including acid-fast bacilli, but with negative results.

May, 1910. The other animal of *first generation A* has remained free of tumor and healthy to date.

Second Generation A.—Nov. 8, 1909. Six market-bought Plymouth Rock chickens, 2 pigeons and 2 guinea-pigs were inoculated with tumor-bits in the muscle of the right breast and also intraperitoneally. The material was obtained from *first generation A* (q. v.).

Jan. 27, 1910. The chickens were reinoculated, this time into the left breast, with material from *second generation B.*

May, 1910. All are still without sign of tumor.

Second Generation B.—Dec. 10, 1909. Three market-bought Plymouth Rock chickens less than 3 months old, and 6 young fowls, 6 months old, of the tumor

stock, were inoculated in both breasts with bits of the growth from the positive fowl of *first generation A.* The 3 market-bought chickens sickened and died within 12 weeks thereafter. In one was no trace of tumor; in the second was a small nodule (0.6 centimeters in diameter) that had undergone myxomatous degeneration; in the third, a small cyst (1.0 centimeters broad), formed by degeneration and liquefaction of tumor-tissue. Some fragments of this tissue still existed at the well-encapsulated periphery of the cyst.

Jan. 17, 1910. Of the 6 fowls from the tumor stock one had developed at this date a tumor mass 1.3 centimeters in diameter. Jan. 27, 1910. The nodule measured 2.5 centimeters and was operated upon for transfer into *third generation A* (q. v.) and for reinoculation of *second generation A.* At this time many cultures were taken and stains made for bacteria but with negative results. During the next few weeks, the mass decreased in size, and no sign of renewed growth was observed until March 21, 1910. April 7, 1910. Growth has of late been rapid and the mass when today cut into measured 4 by 3 by 2 centimeters. Inoculation was done into *third Generation B.* April 18, 1910, the mass operated upon measured 5 by 3.3 by 2.5 centimeters and was approximately egg-shaped, firm and smooth (Plate LXVI, Fig. 1). The wound had healed perfectly.

In none of the other fowls of this generation was growth obtained.

Third Generation A.—Jan. 27, 1910. Five young fowls of mixed breed, 10 market-bought Plymouth Rocks, and 4 chickens, seven months old, of the tumor-stock were inoculated from *second generation B.*

May 9, 1910. To date none have shown tumors except a cock of the tumor-stock in which was noted March 21 a small lump on the left breast. Operation was performed April 7, and inoculation made into *fourth generation A.*

Third Generation B.—April 7, 1910. Twelve market-bought Plymouth Rocks, under 3 months of age, were inoculated in the muscle of both breasts and in the subcutaneous tissue of the left breast with material from *second generation B.* June 1, 1910. In none of these has growth appeared.

Fourth Generation A.—April 7, 1910. Two of the young fowls inoculated on this date received in the right breast material from *third generation A* instead of from *second generation B.* June 1, 1910. Both of these are negative as regards tumor.

So far as tested this new growth in the chicken has proved itself a neoplasm of classical behavior. The peculiarities which it exhibits are those already made familiar through observations on the tumors of the rat, mouse, dog and man. The tissue specificity which has limited its successful transplantation to fowls of the stock in which the primary growth arose is striking, but not more so than the specificity of certain mouse and rat tumors; and this character may in part explain why previous attempts to transplant neoplasms of the fowl have failed.

The tumor is at best so difficult of propagation that no attempts have been made to determine whether it can be transmitted by cell-

fragments, or by cell-free derivatives. For the same reason, the question whether growth takes place entirely from the introduced cells has not been investigated. But there is no reason to suspect that the neoplasm will differ on these points from the better-known tumors of mammals.

It may not be superfluous to point out that such similarity of behavior as has been thus far observed between this avian tumor and those of mammals was, after all, largely to be expected. A graft of mammalian tumor succeeds better in a blood-related individual, and a young one, not because it is tumor, but because it is tissue. The tissue laws here concerned are probably not very different in birds. Nevertheless, the close correspondence in behavior between this avian tumor and the typical mammalian tumors is certainly of interest. Sticker's lymphosarçoma of the dog, which is transplantable to foxes, von Dungern's tumor of the hare, which will also grow in rabbits, both deviate more from the tumor-type as observed in mammals than does this sarcoma of the fowl. At first sight, indeed, the behavior of these unusual growths seems in absolute violation of the laws governing tissues. Yet this is not necessarily true. For in the hybridization of the horse with the ass, of the dog with the wolf, the elements from different species unite in a much more intimate association than exists between a tumor and its host.

SUMMARY.

In this paper is reported the first avian tumor that has proved transplantable to other individuals. It is a spindle-celled sarcoma of the hen, which thus far has been propagated into its fourth tumor generation. This was accomplished by the use of fowls of pure blood from the small, intimately related stock in which the growth occurred. Market-bought fowls of similar variety have shown themselves insusceptible, as have fowls of mixed breed, pigeons and guinea-pigs. The percentage of successful transplantations has been small, but in the individuals developing a tumor its growth has been fairly rapid. Young chickens are more susceptible than adults. The reinoculation of negative fowls has never resulted in a growth.

Throughout, the sarcoma has remained true to type. It is

infiltrative and destructive. Metastasis has been observed once (to the heart). Experiments to determine whether the growth may be transmitted by cell-fragments have not yet been made. Repeated bacteriological examinations have yielded negative results.

In its general behavior, so far as tested, this avian tumor closely resembles the typical mammalian neoplasms that are transplantable.

EXPLANATION OF PLATES.

Plate LXVI.

Fig. 1. Sarcoma. Second generation B.

Fig. 2. Cross-section of same tumor, somewhat enlarged.

Plate LXVII.

Fig. 3. Sarcoma of the chicken from an intraperitoneal growth.

Fig. 4. Sarcoma of the chicken.

Plate LXVIII.

Fig. 5. Invasion of muscle by the sarcoma.

Fig. 6. A metastasis in the heart wall. The lumen shown at the left hand corner is that of a small vein.

FIG. 1.

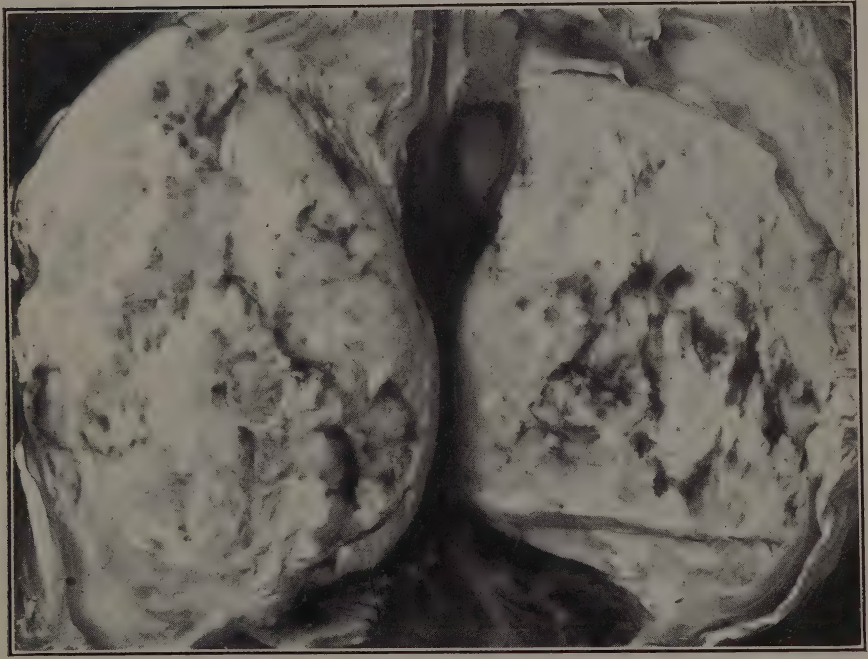

FIG. 2.

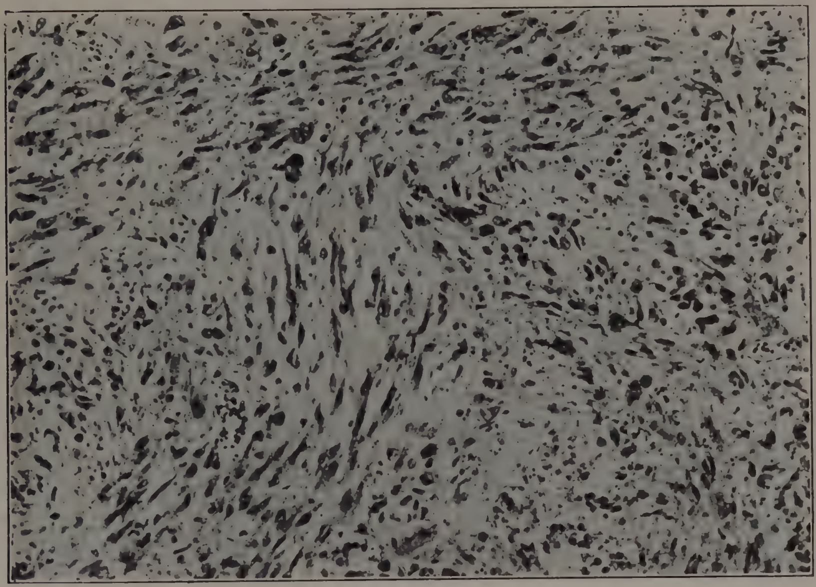

FIG. 3.

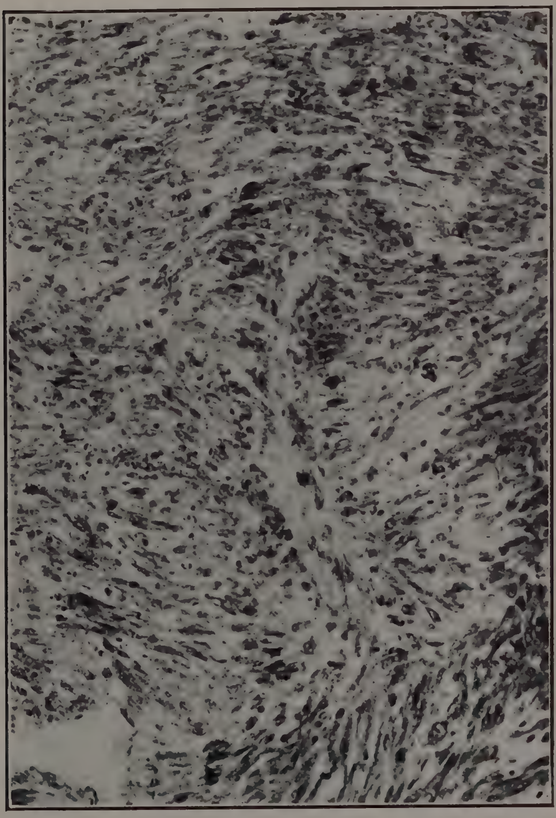

FIG. 4.

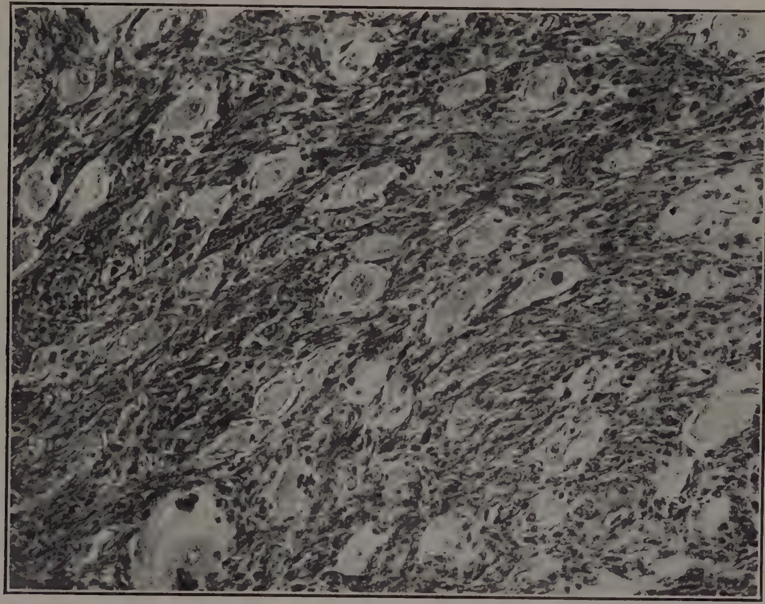

Fig. 5.

Fig. 6.

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A SARCOMA OF THE FOWL TRANSMISSIBLE BY AN AGENT SEPARABLE FROM THE TUMOR CELLS.*

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PLATES XLVII–LII.

A transmissible sarcoma of the chicken has been under observation in this laboratory for the past fourteen months,[1] and it has assumed of late a special interest because of its extreme malignancy and a tendency to wide-spread metastasis.[2] In a careful study of the growth, tests have been made to determine whether it can be transmitted by a filtrate free of the tumor cells. Attempts to so transmit rat, mouse, and dog tumors have never succeeded; and it was supposed that the sarcoma of the fowl would not differ from them in this regard, since it is a typical neoplasm. On the contrary, small quantities of a cell-free filtrate have sufficed to transmit the growth to susceptible fowls.

EXPERIMENTS.

For the first experiments on this point, ordinary filter paper was used, and the ground tumor was suspended in Ringer's solution. It was supposed that the slight paper barrier, which allows the passage of a few red blood cells and lymphocytes, would suffice to hold back the tumor and render the filtrate innocuous. Such has been the experience of other workers, with rat, mouse, and dog tumors. But in the present instance characteristic growths followed the inoculation of small amounts of the watery filtrate, or of the fluid supernatant after centrifugalization of a tumor emulsion.

These results led to more critical experiments, which will be here detailed. Tumors of especially rapid growth, and young, well-grown fowls of the variety in which the tumor originally occurred, namely, the barred Plymouth Rock, were used throughout.

* Received for publication, February 9, 1911.

[1] Peyton Rous, *Jour. Exper. Med.*, 1910, xii, 696.

[2] Peyton Rous, *Jour. Am. Med. Assn.*, 1910, lv, 1805; 1911, lvi, 198.

Experiment I.—Tumor material from the breast of chicken 92 (tumor generation, 6th A) was ground with sterile sand, suspended in a considerable bulk of Ringer's solution, and shaken for twenty minutes in a machine. The sand and tumor fragments were separated out by centrifugalization in large tubes for five minutes at 2,800 revolutions per minute. Of the supernatant fluid a little was pipetted off and centrifugalized anew for fifteen minutes at over 3,000 revolutions per minute. From the upper layers sufficient fluid for inoculation was now carefully withdrawn. The pure-bred fowls were injected in one breast with 0.2 c.c. of the fluid, in the other with a small bit of tumor tissue. All developed sarcoma at the site of this latter inoculation, and in seven the same growth slowly appeared at the point where the fluid had been injected.

Experiment II.—Tumor from chicken 90 (tumor generation, 6th A) was ground, suspended, and shaken as before. After one centrifugalization the fluid was passed through a Berkefeld filter (No. 2, coarse). Before filtration, it was pinkish yellow, cloudy; afterwards, faintly yellow, limpid. Nine fowls were inoculated with 0.2 c.c. of the filtrate in each breast, and twenty-two more received filtrate in one breast, a bit of tumor in the other. Of the nine, one slowly developed a sarcoma in each breast, and microscopic growths were later found in its lungs. Of the twenty-two receiving both filtrate and tumor, five developed sarcoma where the filtrate had been injected, and these five showed especially large growths from the tumor bit.

The Berkefeld filter employed was afterwards found to be slightly pervious to *Bacillus prodigiosis.* The tumor developing in the fowl injected only with filtrate has been successfully transplanted to another individual.

Experiment III.—The filtrate was similarly prepared except that a small Berkefeld filter (No. 5, medium) was used, impermeable, under the conditions, to *Bacillus prodigiosis.* As before, the filtration was done at room temperature. Fowl 124 (7th generation, A) furnished the material. Twenty chickens were inoculated in each breast with the filtrate, but none have developed tumors.

Experiment IV.—In this experiment the material was never allowed to cool. About fifteen grams of tumor from chicken 140 (7th generation, B) was ground in a warm mortar with warm sand; mixed with 200 c.c. of heated Ringer's solution; shaken for thirty minutes within a thermostat at 39° C.; centrifugalized; and the fluid passed through a filter similar to that used in experiment III. Both before and after the experiment, this filter was tested and found to hold back *Bacillus prodigiosis.* The filtration of the fluid was done at 38.5° C., and its injection followed immediately. In four of ten fowls inoculated into the muscle of each breast with 0.2 to 0.5 c.c. of the filtrate, there developed a sarcoma at one of the points of inoculation; and though the growths required several weeks to appear, their subsequent enlargement was of average rapidity. Pieces removed at operation showed the characteristic structure, and transplantation into other chickens proved successful. Three of the hosts have died, and in two profuse metastases were found. One of the growths furnished the material for experiment V.

Experiment V.—The tumor used resulted from the injection into fowl 180 (8th generation, B) of 0.5 c.c. of the filtrate of experiment IV. Just as in this experiment, the material was ground, suspended, and shaken in the warm, but 300 c.c. of Ringer's solution were used to eleven grams of tumor, and the shaking

was continued only twenty minutes. After centrifuging, the filtration was done at 40° C., through a new Berkefeld filter (No. 5, medium), impermeable, under the same conditions, to *Bacillus prodigiosis.* Ten young hens were inoculated in each breast with 0.5 to 1.0 c.c. of the filtrate; and eleven days later a tumor nodule was just palpable in two of them. One of these growths was at once removed by operation. It took the form of a small raised disc, firm, grayish, and translucent, on the outer surface of the pectoral sheath, presumably at the point penetrated by the injection needle. Its greatest diameter was one and one-half millimeters. In the other fowl the nodule lay deep in the muscle, and seemed little, if at all, larger than that described. In control fowls inoculated with bits of the neoplastic tissue from No. 180, growths measuring from 1.3 to 2.7 cm. in diameter had developed at this time. Twenty-eight days after inoculation, eight of the ten fowls given the filtrate showed tumor nodules, some of them still very small.[3]

Thus the tumor resulting from injection of a filtrate itself furnished material capable of producing tumors after injection.

The importance of the above results depends on the characters of the growth employed. These will now be discussed in detail.

STRUCTURE AND MODE OF GROWTH.

The original tumor was found in the subcutaneous tissue of the breast of an adult, pure-bred hen. The other individuals of the small stock were healthy; and though susceptible normal chickens and chickens with the tumor have been kept together for long periods, no instance of spontaneous transmission of the growth has occurred. The characters of the original mass have been detailed elsewhere.[4] A more general description of the tumor as it has appeared on transplantation will here be given.

Histologically the growth has always consisted of one type of cells, namely, spindle cells, usually in bundles, with a slight vascularizing framework (figure 1). Cell division is usually by amitosis, but mitosis is frequent. Small giant cells, due to the division of the nucleus without corresponding fission of the cytoplasm, are occasionally seen, especially about regions where the growth is degenerating. There is considerable variation in the size of the cells and in the staining qualities of their nuclei, but the growth has not changed in general histological character during its propagation

[3] Later work in this laboratory, by Dr. James B. Murphy, has demonstrated that the tumor can be transmitted by means of the dried and powdered neoplastic tissue, kept at room temperature for many days. The tumors resulting from its injection do not appear for several weeks.

[4] Peyton Rous, *Jour. Exper. Med., loc. cit.*

to the tenth tumor generation. Tumors of the eighth generation, resulting from a cell-free filtrate, resemble the original, except that their cells are plumper, less regular in size, and much more invasive in tendency (figure 2). Figure 3, a drawing from the edge of a growth in the breast that followed injection of a filtrate (experiment IV), illustrates the replacement of striated muscle fibres by tumor cells. Here most of the neoplastic cells are oval, as yet undifferentiated to the spindle form seen in older portions of the growth. Under some conditions, the cells become widely separated by mucigenous ground substance (figure 4). The picture is always that of a spindle-celled sarcoma or myxosarcoma.

Following implantation of a bit of the neoplastic tissue into the breast muscle of a susceptible fowl, a circumscribed nodule shortly becomes evident. It is very firm and definite on palpation, but on section proves unencapsulated, though distinct in appearance from the normal tissue. In general, the neoplastic tissue is gristly and grayish white, with a fine striation on the cut surface. Less often, it is soft, grayish pink, semitranslucent, and friable, or even gelatinous. In the last instance, it may contain much true mucin. As the mass grows large, its scanty, thin-walled blood vessels prove insufficient, and at its center a wide-spread coagulative necrosis, or cystic change takes place, the latter not infrequently as the result of hemorrhage. The cysts are filled with serous or ropy fluid, often colored with blood pigment; and polypoid extensions into them of the tumor are not rare. Continuing to grow, the mass extends to the muscle-sheath and perhaps through this to connective tissue and skin. Infiltrating the latter, it may spread rapidly *en cuirasse;* but ulceration is seldom seen. Soon the whole of the inoculated breast is occupied by a bulging, rounded, firm growth (figure 5); and the host rapidly emaciates, becomes cold, somnolent, and dies. In many cases the viscera, especially the lungs, heart, and liver, are the site of discrete metastases, gristly and firm, like the primary growth. Those on the surface of the liver may be umbilicated (figure 6).

TUMORS RESULTING FROM A FILTRATE.

The tumors which result from the injection of a cell-free filtrate take much longer to appear. The inoculation into the breast muscle

of a bit of tumor tissue one millimeter in diameter may give rise in the course of a week to a growing nodule 1.5 centimeters broad; whereas, following the injection of a filtrate, not the slightest trace of tumor is palpable for from ten days to three weeks. Then one or two minute, shotty bodies can be felt, and soon the characteristic mass develops. At first it is ovoid or spherical in shape, just as though it had arisen from an introduced bit of neoplastic tissue. In one case a flat, irregularly branching mass, flame-shaped, so to speak, developed in the sheath of the pectoral muscle at the point injured by the injecting needle. Many of the injected fowls in which no growth appeared in the breast muscle have been carefully examined at autopsy for tumors elsewhere, but none have been found. Some with a growth in the breast have developed after a time others in the viscera, probably the usual metastases, to judge from size and distribution. Careful note has been kept as to whether the tumors resulting from a filtrate injection grow more slowly than usual. This has been found to be the case. Following their tardy appearance, a considerable proportion of them grow slowly as compared with control tumors resulting from implantation.

INFLUENCE OF THE HOST.

The stock in which the original tumor occurred consisted of fowls of one pure-bred variety, the barred Plymouth Rock. The first transplantation was made to chickens from the same setting of eggs as the individual with the tumor, and the next successful one to less closely related members of the same stock. Bits of the growth were placed in the breast muscle by means of a trocar, a procedure adopted as the routine. Repeated unsuccessful attempts were made to transfer the growth to chickens resembling the tumor stock, and of similar variety, but obtained from another source, and probably not pure-bred (chart 1). Attempts at this time to transfer it to chickens of another variety and to pigeons and guinea pigs also failed. Recently the sarcoma has increased in malignancy and has gained the power to grow in chickens of other kinds. Yet in them it develops slowly, or long remains stationary; and it has never been successfully transmitted to other species, although pigeons, ducks, rats, mice, guinea pigs,

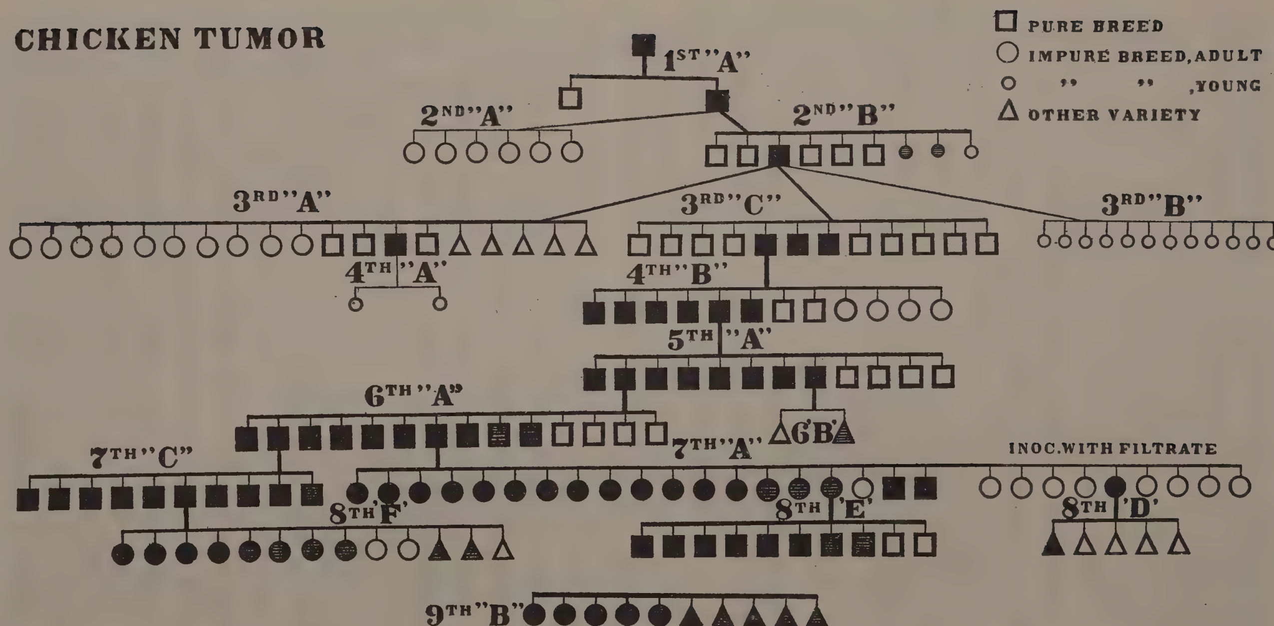

Chart I. This chart of the first eight generations of the sarcoma illustrates the influence of the variety of the host. The blackened symbols represent those hosts in which the tumor grew, the cross-barred ones those in which it appeared, but remained stationary, or retrogressed. The fowls used were barred Plymouth Rocks of pure breed, barred Plymouth Rocks bought at random and hence presumably not pure-bred, and other chickens of heterogeneous sort and appearance. All were inoculated with bits of the tumor, except nine individuals of 7th generation A, which received portions of a Berkefeld filtrate. A number of the series of the 7th and 8th generations are omitted from the chart, because in them the conditions were irregularly modified. The chickens of 9th generation B were inoculated with material from a member of one of these series. Here the importance of the host's variety is still evident, though the cross-barring of the symbols now means that the tumor grew slowly, not that it remained stationary or retrogressed.

and rabbits have recently been inoculated. At present it grows in 80 to 100 per cent. of barred Plymouth Rock fowls and is especially active in young ones. Retrogression of a developed nodule, fairly frequent at first, is now rare, save when the host is sick. Intercurrent illness of the host may check the further development of a nodule two or three centimeters in diameter, and may even cause its retrogression (chart 2). Should the host's health return, the tumor may reappear and grow rapidly. Retrogression in the healthy host confers resistance, so that further inocluation with active material does not result in growths. A fowl which has once failed to develop a tumor usually proves resistant on later inoculation. Resistance, therefore, is both induced and, apparently, natural also.

EFFECTS OF TRANSPLANTATION.

The original tumor had been under observation for two months before it was brought to the laboratory, and had grown slowly during that period. With repeated transmission the rate of growth, as well as the percentage of successful transplantations, has increased; and the period which elapses between implantation and the appearance of the new sarcoma as a palpable mass has been reduced from about four weeks to four or five days. The tumor obtained from the first inoculation required seventy-one days to reach a size of 5.0 by 3.3 centimeters, and to affect seriously the health of the host. But in later generations, produced by a similar method of inoculation, the appearance and development of the tumor have become progressively more rapid. Growths ten or twelve centimeters in length by six in width are now found three weeks after inoculation of a bit of neoplastic tissue, two millimeters in diameter; and often death of the host ensues within twenty-six to thirty days, all told.

Repeated transplantation has also greatly increased the frequency, extent, and rapidity of metastasis formation. The death of the original fowl was hastened by a successful intraperitoneal implantation with its own growth, and the autopsy did not disclose any nodule suggesting a metastasis. The tumor fowl of the first generation was killed after seventy-two days, and had, in its heart, one small secondary growth. Metastasis did not take place in the second

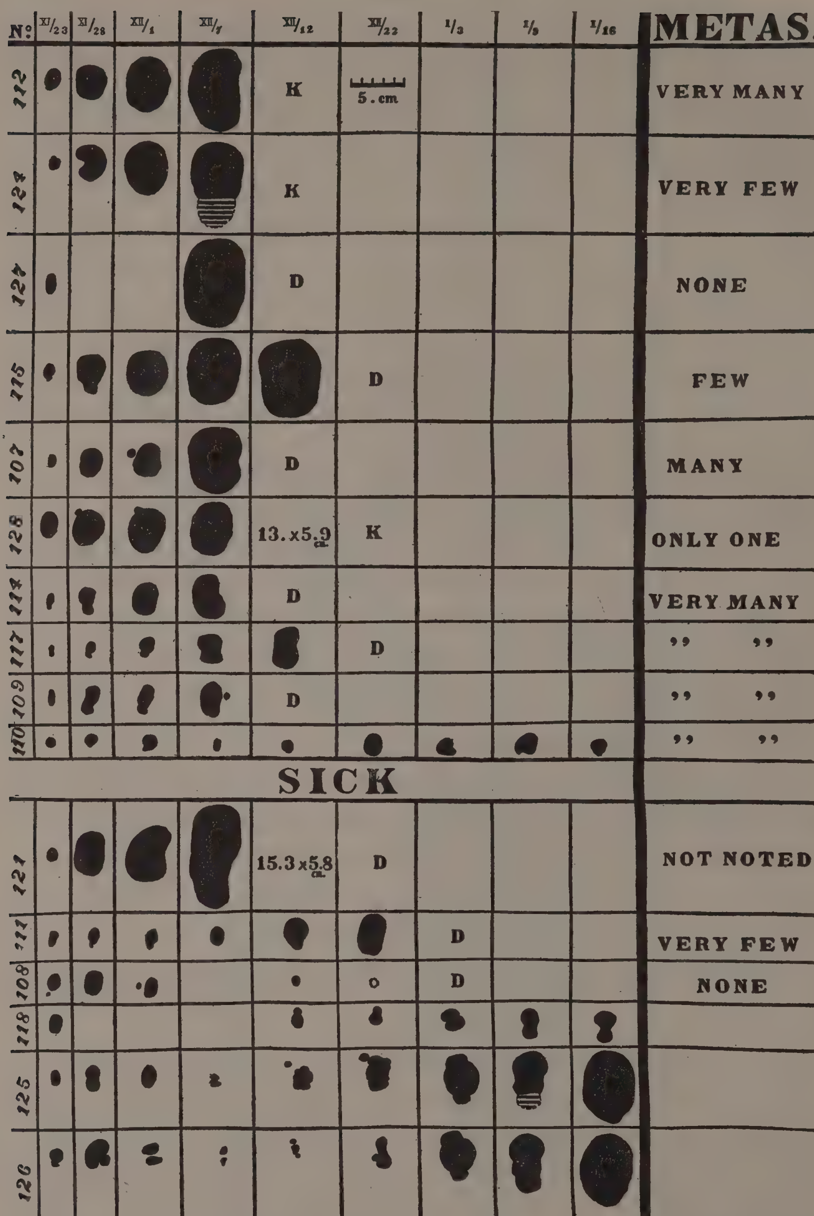

CHART 2. The effect upon the tumor's growth of intercurrent illness of the host. The figures in the first vertical column are the serial numbers of the

fowls, those in the first transverse column refer to the dates on which the tumors were measured. In the last vertical column, record is made of the visceral metastases at autopsy. "D" or "K" indicates that the fowl died or was killed.

The fowls (7th generation A) were all inoculated with the same material, and the first measurements were taken eight days later. Within two weeks, six of the hosts had fallen ill of an intercurrent disease, characterized by rhinitis, conjunctivitis, and marked depression and emaciation. Tumors had already developed in all six, but now retrogressed in four, completely disappearing in two. The fowls were placed under better conditions and three recovered health. In two, the tumor reappeared and grew rapidly.

tumor generation; but in the third it was frequent; and in the later ones it has been present in a majority of hosts dying of the growth. In the seventh generation the average time required after inoculation for the dissemination and development of secondary nodules was approximately half that demanded in the third. Metastasis takes place first and most often to the lungs (figure 7). In the earlier generations the heart was especially effected (figure 8), though nodules were found in the liver, much less frequently in the kidneys (figure 9), and occasionally in the intestine, mesentery, pancreas, gizzard (figure 10), gall-bladder, and lymph-glands. Of late the heart has held fewer nodules, and they have been frequent in the liver. The spleen was exempt from them until the seventh generation, when in two cases out of twenty-five it contained nodules. In these two cases the tumor showed itself in other ways especially malignant. The spleen has since been often affected.

MODE OF METASTASIS FORMATION.

The question as to how metastasis of the chicken sarcoma takes place has great importance. For it might be supposed that the agent which suffices, independent of the cells, to transmit the tumor to new individuals would itself cause secondary masses in the host. Perhaps sometimes it does act alone to produce such masses, though we have met no instance in which this can be affirmed. On the contrary, the findings all indicate, as with the sarcomata of man, that the metastases result from a distribution of tumor cells, usually by way of the blood. As has been said, the relations of the sarcoma to the blood stream are very intimate; quite large vessels walled with only a layer of endothelium exist within the growth, and in their

neighborhood cell proliferation is at its height. Instances in which the neoplastic tissue has penetrated a vessel wall are frequent (figure 9), and sometimes a strand of the growth, quite bare of endothelium, extends for a considerable distance in the blood stream (figure 8). The secondary growths in the viscera are first evident as small, approximately spherical groups of cells with a blood-vessel in the midst, occluded by tumor tissue (figure 7). The cells are of one type, in active mitotic and amitotic division, unsurrounded by inflammatory reaction. This is true of metastases consisting of only three or four cells. To recognize a single neoplastic cell lodged in a capillary has thus far proved impossible because the morphology of the single cell does not sufficiently identify it; yet what would appear to be such emboli are frequent in the pulmonary tissue. The question of the transplantability of the tumor cells has been settled in the affirmative by an examination of grafts of the neoplastic tissue removed shortly after implantation.

Metastasis by way of the lymph-stream occasionally occurs: the glands along the great vessels above the heart have been found enlarged and entirely replaced by sarcomatous tissue. Contact metastasis is very frequent. For example, a mass in the breast may penetrate the sternal membrane and give rise to nodules on the liver surface opposite. In the viscera the tumor preserves its histological character, but its arrangement is influenced to a certain degree by preëxisting structures. In the lung the pattern of the alveoli may be perpetuated in tumor cells, as is true also of the striped muscle (figure 3). The skin may be infiltrated and tightly stretched. Frequently the growth recurs in wounds made to remove subcutaneous grafts, and here its presence need not greatly impede healing. The early stages in the development of a sarcoma caused by a cell-free filtrate are difficult to obtain. When such a growth becomes palpable, it is already one to two millimeters in diameter, and histologically no more enlightening than a metastasis or a graft of the neoplastic tissue would be.

THE TUMOR CELLS ARE TRANSPLANTABLE.

A study of many grafts removed at short intervals from the connective tissue has shown conclusively that the inoculation of a

bit of the sarcoma into a susceptible fowl results in an actual transplantation of the neoplastic cells and growth from them. For the first two or three days after implantation, the graft is unattached to the host tissues, but then it unites with them, is vascularized, and begins to enlarge and to invade the surrounding parts (figure 11). Usually a few small mononuclear cells (lymphocytes) collect at its edge, but no other cellular reaction follows that is due to the initial trauma. Indeed, about metastases a cellular reaction is often completely absent. Unless the graft is very small its central part dies before vascularization can take place. There remains, however, a living periphery distinct from the normal tissues of the host, and soon this is vascularized and strands of the spindle-shaped cells can be seen growing out from it. The findings do not in the least suggest that the tumor is transmissible apart from its cells. Certainly it is transplanted easily and is, at present, best propagated by this means.

The death of grafts of the tumor in fowls with a natural or acquired resistance takes place in one of two ways. The implanted tissue may fail entirely to be vascularized, and its death results after some days, during which a zone of living cells persists at its periphery (figure 12). Or it may be vascularized and grow for a brief period, dying at last in the midst of an accumulation of lymphocytes. The first process is seen especially in regions poor in connective tissue and blood-vessels, and may occur there even in susceptible hosts. The second process is the one found at the edge of retrogressing tumors. A more detailed account of the fate of early grafts has been reserved for another paper. The findings here briefly described are those made familiar to tumor workers by a study of rat, mouse, and dog tumors.

Cultures from the growth upon many media have repeatedly been taken; but with the exception of a large post-mortem bacillus once obtained, they have remained sterile as regards bacteria. Portions of the filtrate and fresh smears from the tumor surface have been examined with the dark-field microscope, but neither this nor the various histological procedures applied to the neoplastic tissue has disclosed anything which can be recognized as a parasitic organism.

DISCUSSION.

It is evident from the foregoing description that our tumor of the fowl possesses to a marked degree those characters of morphology and behavior which distinguish the true malignant neoplasms, especially the sarcomata. It is formed of a single type of cells, only slightly differentiated, resembling young connective tissue cells, and possessed of an enormous proliferative energy which is exercised to the detriment of the surrounding tissues and eventually of the entire host. Growth takes place through infiltration and replacement of normal structures, as well as through expansive enlargement. Metastasis by way of the blood stream is common, rarer by the lymphatics; and, to judge from histological evidence, the transportation, lodgment, and growth of tumor cells is wholly responsible for the secondary nodules. Indeed, a general histological study of the sarcoma would not lead one to suspect that it can be transmitted by another means than a transplantation of cells. When a small bit of the neoplastic tissue is placed in a new and susceptible host, most of its cells survive, are vascularized, and by their proliferation give rise apparently to all of the growth. In a resistant host, the graft soon dies and no tumor follows. One would suppose that the sarcoma developed only "aus sich heraus," to use Ribbert's phrase. But histological pictures are not decisive upon this point. Since the growth is transmissible by a cell-free filtrate, it seems not unlikely that in its neighborhood the connective tissue cells of the host undergo a neoplastic change.

A feature of the transmissible tumors, which has largely drawn the attention of cancer workers and has modified current theories of cancer origin, is their striking dependence for a successful transplantation on the character and condition of the individual host. It is a dependence similar to that shown by transplanted normal tissue, and apparently the same laws largely influence both. This trait of tumors is illustrated exceptionally well by the chicken sarcoma. During a considerable period, it could be propagated only in fowls of precisely the sort in which the original growth occurred (chart 1); and even now it succeeds best in these. It has never been successfully transmitted to birds of other species, or to mammals. Young fowls are the most favorable hosts; and healthy,

well-nourished ones proves more susceptible than the thin and ill. Indeed, intercurrent illness of the host may cause the sarcoma transiently to disappear (chart 2).

The above traits have figured largely in current discussions on cancer etiology, and most of them have been regarded as evidence against a specific cause for the disease, extrinsic of the cells. Such evidence is void, now that a growth has been found possessing the traits mentioned, yet transmissible independently of its cells. This fact, and not the problem of how to classify the growth, merits attention. Nevertheless, a passing reference should perhaps be made to the ill-defined group of pathological products called granulomata, with which this neoplasm of the fowl may by some be classed, owing to its transmission by an agent separable from the tissue cells. None of the granulomata has the tumor characters, and none is known to be transplantable. The present growth fails to resemble any granuloma thus far described; whereas it fulfills all the conditions for identification as a tumor.

The first tendency will be to regard the self-perpetuating agent active in this sarcoma of the fowl as a minute parasitic organism. Analogy with several infectious diseases of man and the lower animals, caused by ultramicroscopic organisms, gives support to this view of the findings, and at present work is being directed to its experimental verification. But an agency of another sort is not out of the question. It is conceivable that a chemical stimulant, elaborated by the neoplastic cells, might cause the tumor in another host and bring about in consequence a further production of the same stimulant. For the moment we have not adopted either hypothesis.

The ultimate significance of these unusual findings can hardly be well discussed until more data are obtained through experiment, especially through carefully devised experiment with the tumors of other species of animals. For it is quite possible that the failure to separate from these growths an agent causing them may be traceable to some interference with the conditions under which this supposititious agent can exist alone, or reproduce the growth in new hosts. Work along the line indicated is under way in this laboratory.

EXPLANATION OF PLATES.

Unless otherwise indicated, the sections are stained with Delafield's hematoxylin and eosin. In each case the tumor generation and series are given, followed by the number of fowl from which the specimen came; thus, 7th A, No. 117.

Plate XLVII.

Fig. 1. An area in the original growth. Necrosis is present at one side.

Fig. 2. 8th B, No. 177. Part of a tumor in the left breast, resulting from the injection of the filtrate of experiment IV. The muscle fibres are largely invaded and replaced by tumor cells. Methylene-blue and eosin.

Plate XLVIII.

Fig. 3. 8th B, No. 177. A drawing which further illustrates the invasion and replacement of muscle fibres by tumor cells. The preparation is from the same growth as Fig. 2.

Fig. 4. 8th B, No. 173. Myxomatous growth caused by the filtrate of experiment IV.

Plate XLIX.

Fig. 5. 5th A, No. 82. Growth in the left breast of a chicken, resulting from the implantation of a small bit of tumor tissue fifty-two days previously. The skin and connective tissue covering have been removed. The length of the mass is fourteen centimeters. In the right breast is a nodule that resulted from injection of the fluid supernatant after centrifugalization of a tumor emulsion.

Fig. 6. 7th A, No. 117. Metastases from a growth in the left breast. The chest, and abdominal wall, and about half of the breast tumor have been cut away to expose the viscera. The lungs, much enlarged, are crowded with discrete tumor nodules. The metastases on the surface of the liver are umbilicated and surrounded by a zone of dilated blood-vessels. The duration of the disease was thirty-seven days.

Plate L.

Fig. 7. 6th A, No. 102. A small metastasis in the lung, with an occluded blood-vessel at its center. The tumor cells have only short, blunt processes, a variation that is not infrequent. The picture is complicated by the presence of many nucleated red cells.

Fig. 8. 4th B, No. 63. Growth of a tumor of the heart wall into the ventricular blood. A tongue of myxomatous tumor here extends between two trabeculae of heart muscle. The dark mass at its end consists of nucleated erythrocytes. The transverse rent in the heart muscle above the tumor is an artefact.

Plate LI

Fig. 9. 4th B, No. 63. Kidney. Extension of the tumor through the wall of a vein.

Fig. 10. 7th A, No. 116. Margin of a metastasis in the muscle of the gizzard. At one corner some uninvaded tissue is seen. Note the complete absence of any cellular reaction about the tumor.

PLATE LII.

FIG. 11. A graft of the sarcoma removed with the surrounding tissue four days after implantation in a susceptible host. Already it has united at two points with the host tissue and vascularization is in progress, though too late to prevent necrosis at the center of the graft.

FIG. 12. A similar graft removed from a resistant host nine days after implantation. Despite the long period, the graft is joined to the host only by two thin strands of connective tissue shown at either end. It is unvascularized and necrotic save for a thin peripheral zone of the characteristic cells, which is considerably infiltrated by lymphocytes.

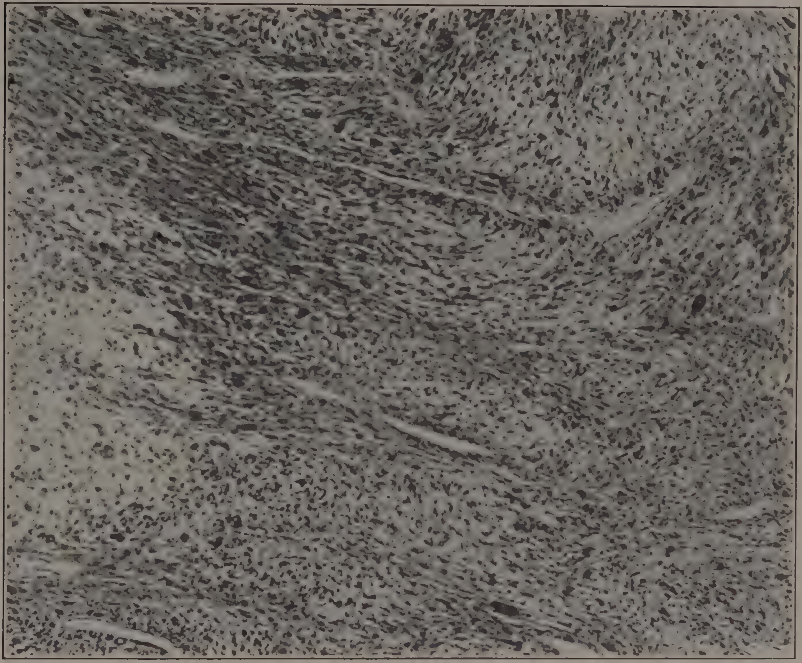

Fig. 1.

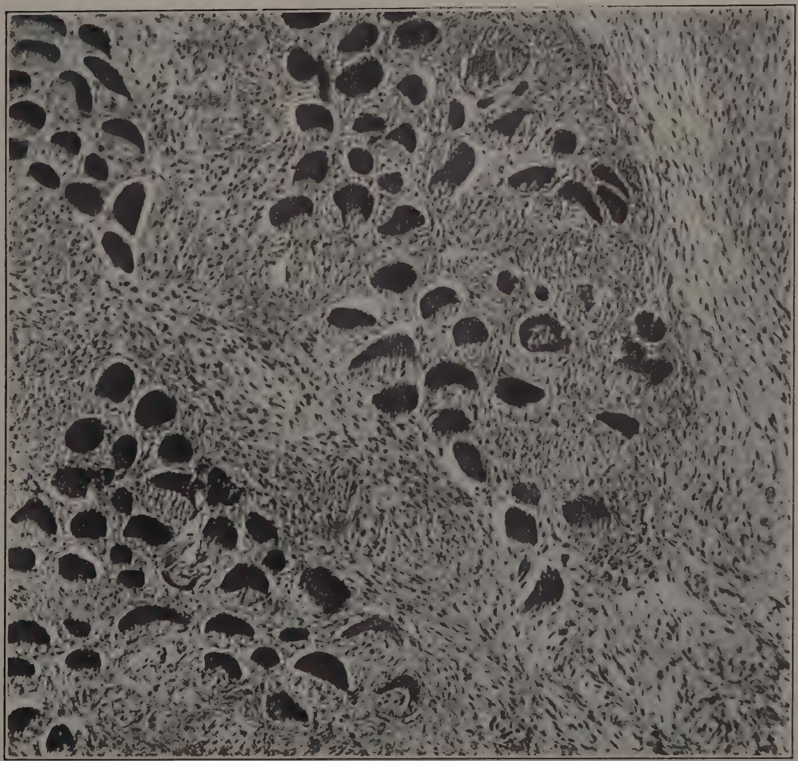

Fig. 2.

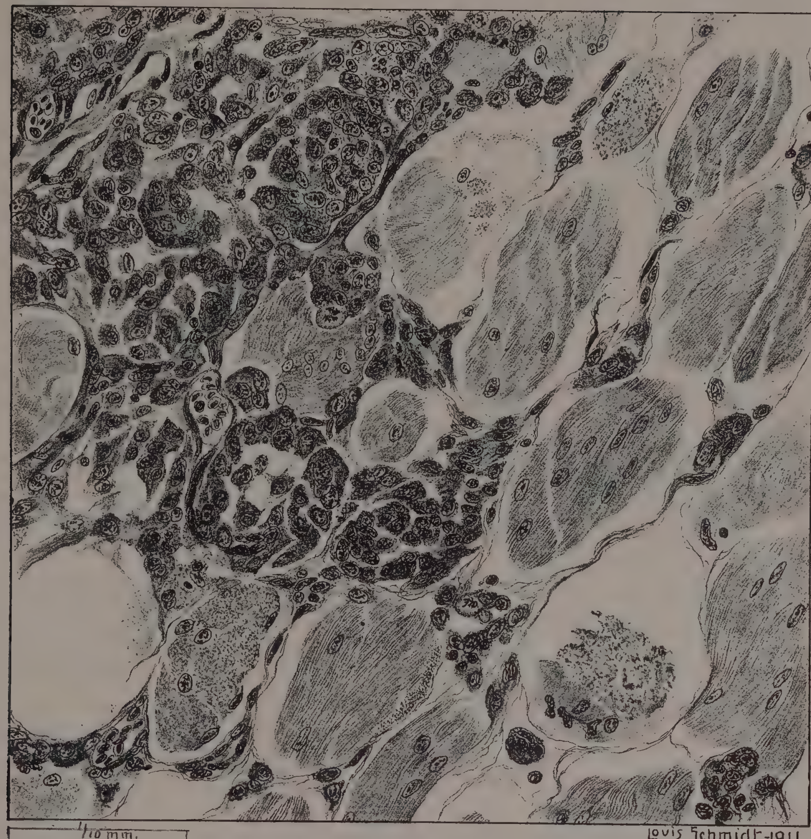

FIG. 3.

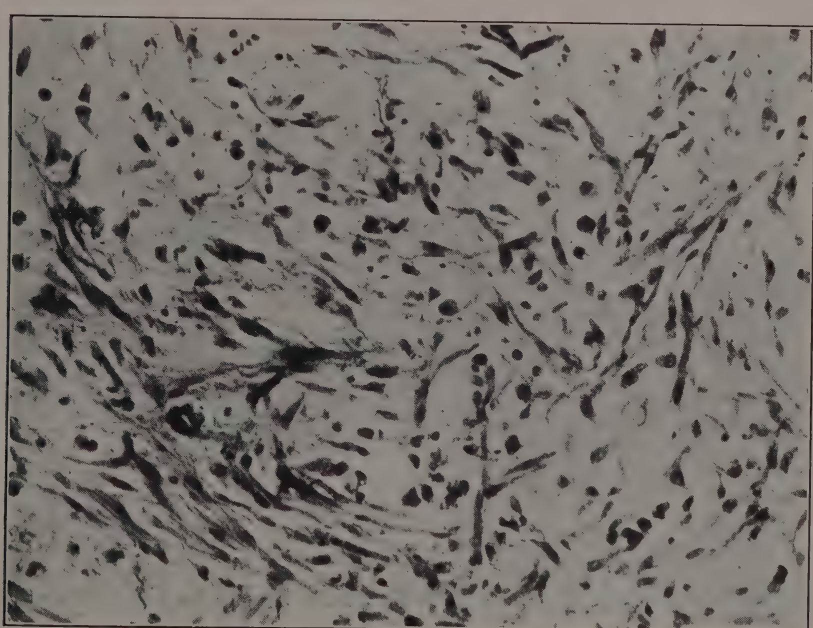

FIG. 4.

FIG. 5.

FIG. 6.

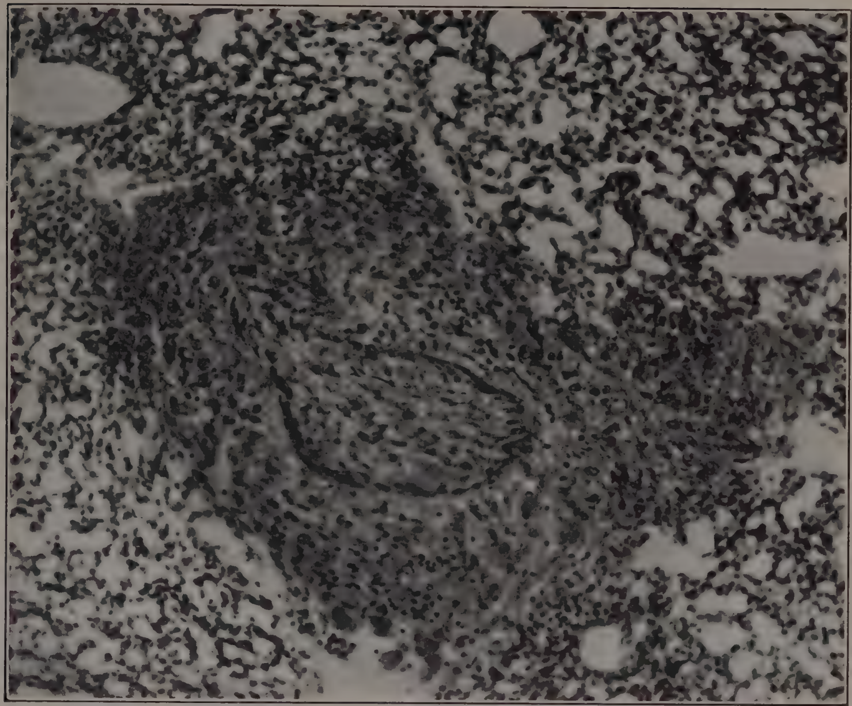

Fig. 7.

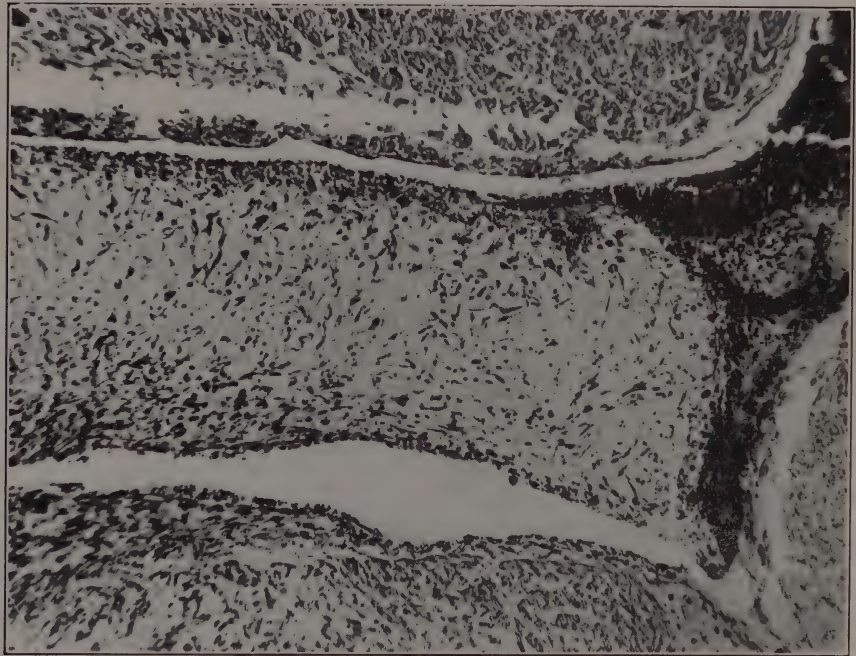

Fig. 8.

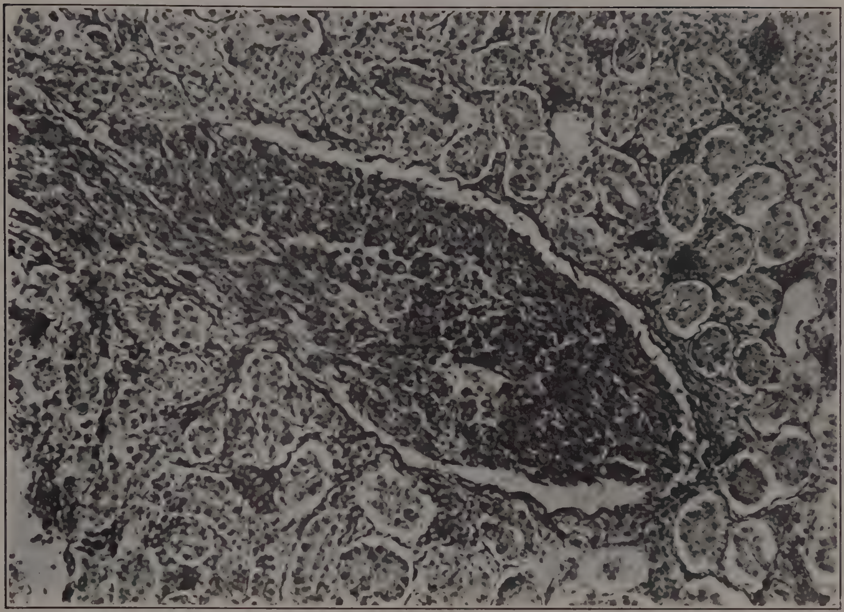

Fig. 9.

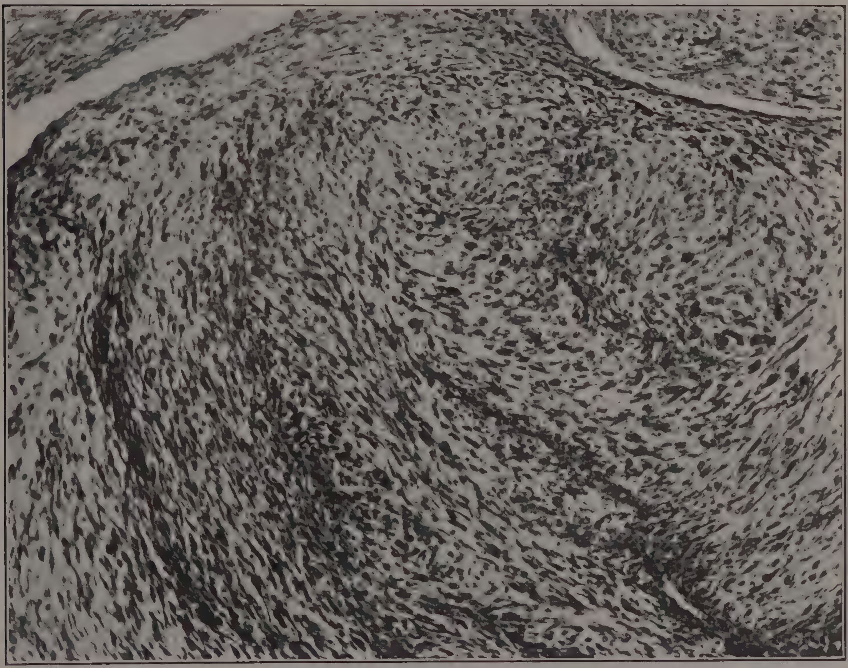

Fig. 10.

FIG. 11.

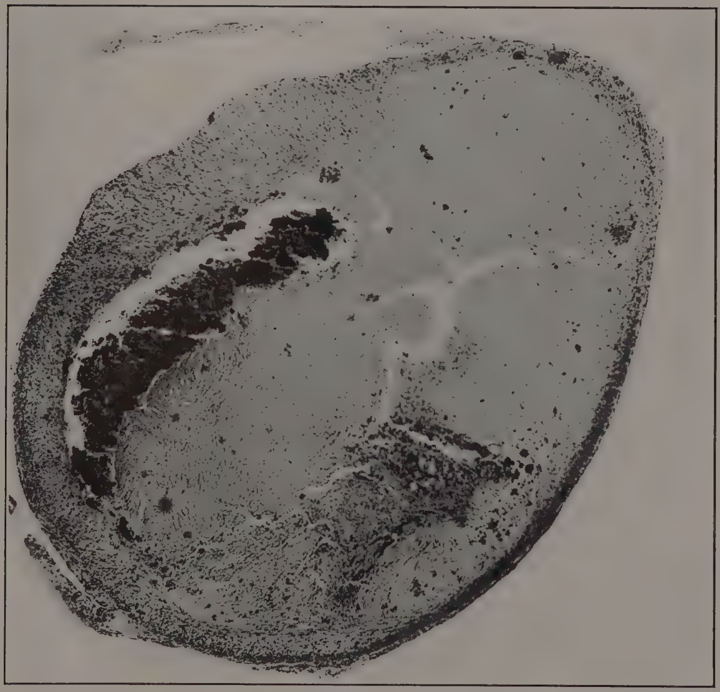

FIG. 12.

TUMOR IMPLANTATIONS IN THE DEVELOPING EMBRYO

EXPERIMENTS WITH A TRANSMISSIBLE SARCOMA OF THE FOWL*

PEYTON ROUS, M.D., AND JAMES B. MURPHY, M.D.
NEW YORK

The fate of tumor cells implanted in the developing embryo has from several points of view a considerable interest. The superficial similarity between neoplastic cells and the cells of embryonic tissue is striking; and it has given rise in part to a widely discussed theory of tumor origin. While this theory well explains a certain class of congenital growths, its more general applicability is questionable. But there is no doubt that the parallel study of embryonic and neoplastic cells can throw much light on important problems of growth.

MATERIAL USED

The implantation of tumor in developing embryos does not seem to have been accomplished heretofore, despite the evident bearing of such an experiment on the supposition that all tumors are of congenital origin. Indeed, it is most difficult with mammalian material, although not impossible, as we have found. After laparotomy of the pregnant rat under ether, a fine, hollow needle is run for some distance in the uterine wall, and thence into the amniotic sac and embryo. When the needle is withdrawn after the injection, there is usually little loss of amniotic fluid. But the percentage of failures from one cause and another is high; and since it is necessary for the survival of the injected embryo that it be large, birth takes place before the tumor has opportunity to grow.

The difficulties are less with developing hen's eggs and a transmissible avian sarcoma.[1] But before describing

* From the Laboratories of the Rockefeller Institute for Medical Research, New York.

1. Rous: Jour. Exper. Med., 1910, xii, 696.

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our results mention should be made of a unique and significant finding recently obtained with this tumor, and now repeatedly observed. It is transmissible to new hosts by means of a filtrate free of the tumor cells.[2] The tumor has also been successfully transmitted in adult fowls by means of dried material kept at room temperature for three days. Nevertheless the growth metastasizes by the proliferation of transported cells and its tissue is in the strict sense transplantable.[3] Indeed, it exhibits to a special degree, not merely a few, but all the main features by which malignant neoplasms are characterized.

METHOD

For the inoculation of the sarcoma into developing chick-embryos, we have slightly modified a method devised by Peebles[4] for another purpose. A small window is cut in the egg-shell, a smaller one torn in the shell membrane, and through a needle a minute portion of the finely divided tumor is injected into the chick, now plainly visible. The window in the shell is then closed, preferably with the piece first cut out, and is sealed with strips of moist shell membrane. When the operation is conducted carefully and with sterile instruments, many of the embryos continue to develop.

The eggs used were laid by pure-bred fowls of the variety most susceptible as hosts for the tumor, namely, barred Plymouth Rocks. At the time of inoculation, the embryos were from six to sixteen days old. Most of our observations thus far have been on tumors in the embryonic membranes, because here even small growths are sharply defined and easily accessible. Furthermore, it is difficult to inoculate the embryo without at the same time inoculating the membranes pierced by the needle.

GROWTH IN THE EMBRYO

When an egg is opened on the sixteenth day of incubation, and a week after a successful tumor inoculation, one observes a slight puckering and opacity where the needle was thrust through the outermost embryonic membrane (fused chorion and allantois). From without no tumor is visible, but on cutting and turning over the

2. Rous: Transmission of a Malignant New Growth by Means of a Cell-Free Filtrate, The Journal A. M. A., Jan. 21, 1911, p. 198.

3. Rous: Metastasis and Tumor Immunity with a Transmissible Avian Neoplasm, The Journal A. M. A., Nov. 19, 1910, p. 1805.

4. Peebles, F.: Arch. f. Entwcklngsmechn., 1908, vii, 405.

membrane, there is usually found on its inner surface at the scarred spot, a sessile nodule like a flattened sphere, of which practically the whole bulk projects into the allantoid cavity. The mass is firm, yellowish or grayish white, nearly opaque, with extremely well-defined contour. Dilated blood-vessels converge to, and course over it. The tumor, after only seven days' growth, may measure 1.2 cm. in diameter, and to see so large a mass dependent on a filmy membrane for support and vascularization is extremely striking. Scattered near by are perhaps a few small nodules, 0.1 to 0.2 cm. broad, and sometimes others lie in the deeper membranes. In the embryo tumor masses are found along the track of the needle. Our observations of this sort have been few as yet. In the best instance found thus far—that of a nineteen-day embryo inoculated a week previously—a large mass lay in the chest wall and projected inward toward the heart. Sessile on this organ was another mass, and two smaller ones were embedded in the liver substance. All were invading the surrounding tissue.

We have once found a "rice-body" free in the fluid between the membranes. It was made up entirely of living and dividing tumor-cells, was unprovided with blood-vessels, and was quite bare of endodermal or ectodermal covering.

GROWTH CAUSED BY A CELL-FREE FILTRATE

Some Ringer's solution in which ground tumor had been suspended and shaken, was passed through a Berkefeld filter impermeable, under the same conditions, to *Bacillus prodigiosus,* and small portions of the filtrate were injected into developing eggs. In one, after the lapse of thirteen days, small, discrete tumor nodules were found on the membranes. A microscopic examination of these well demonstrated the specific action on connective tissue of the agent causing the growth. The fluid had been injected into an extraembryonic body cavity at a time when it was lined by mesoderm, and the tumors occurred as localized growths in this mesodermal lining. Some of them, like the mesoderm about them, were still bare of any covering on one surface. During our experiments the ectoderm and endoderm have often been injured and presumably brought into association with the agent causing the growth, but they have never undergone a neoplastic change.

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HISTOLOGY OF THE GROWTHS

The tumors obtained on implantation resemble microscopically those in adult fowls, except that the cells lie in a loose reticulum and are often of most attenuated spindle form. Slight variations occur in the morphology of the different growths, owing probably to variations in the implanted material. But the cells are of one type, growing in a diffuse mass; and a cellular reaction is quite absent from the normal tissue round about. In general appearance the tumor cells are markedly different from those of the normal embryonic connective tissue next to them.

SUSCEPTIBILITY OF THE EMBRYO

Some embryos are naturally resistant, as are some adult fowls, and tumor implantation in them fails of success; but in most, the growth greatly surpasses that seen in adult hosts. This refers only to tumors in the embryo's membranes, since the implantations in the embryo's body have been as yet too few for a generalization. There the findings will probably be the same. It is well known that young animals form more favorable hosts for transmissible tumors than do old. One of us has recently investigated the point further by inoculating with tumor many new-born rats and mice. These showed themselves much more susceptible than control animals three-fourths grown.

Sixty-Sixth Street and Avenue A.

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THE RÔLE OF INJURY IN THE PRODUCTION OF A CHICKEN SARCOMA BY A FILTERABLE AGENT *

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NEW YORK

The tumor produced by the injection of a Berkefeld filtrate of our transmissible chicken-sarcoma is first noticed as a minute nodule at some point in the track of the injecting needle;[1] and only a small proportion of the fowls injected develop the growth. On the other hand, when the causative agent has been introduced in the form of dried and powdered tumor-tissue, suspended in Ringer's solution, the sarcoma appears as a more or less diffuse mass at the site of injection; and it is found in many of the fowls. These facts have led us to suppose that the filterable causative agent requires for its action a cell-derangement or proliferation, such as the needle-prick or the presence of dried tissue induces. Experiment shows that this is indeed the case.

A number of susceptible fowls were injected, in one "breast" with a large quantity of active filtrate, in the other with an equal quantity of filtrate to which was added a little sterile, washed, diatomaceous earth. Podwyssozki[2] has shown that diatomaceous earth by its mechanical action produces in guinea-pigs a reactive proliferation of the connective-tissue elements; and our controls prove this to be true in chickens as well. The chickens injected with the Berkefeld filtrate were examined every week. Many of them developed a growth where both filtrate and diatomaceous earth had been injected, and in a lesser number a tumor also resulted from the filtrate alone. But in the one instance the tumor regularly arose as a diffuse mass, owing, as examination showed, to a simultaneous proliferation from

* An article somewhat introductory to this one appeared in The Journal, June 1, 1912.

* From the Laboratories of the Rockefeller Institute for Medical Research.

1. Jour. Exper. Med., 1911, xiii, 397.
2. Podwyssozki, W.: Beitr. z. path. Anat., 1910, xlvii, 270.

many foci; whereas in the other it slowly appeared as a discrete, small nodule in the needle-track.

The effect of the filterable agent when injected into the blood-stream was now taken up. It was found that when a large quantity of an active Berkefeld filtrate, free of foreign particles, was injected into the circulation, a tumor seldom resulted (four cases among seventeen). The sarcoma arose more frequently when a little diatomaceous earth had been added to the filtrate, seven out of twenty fowls then developing a growth. Apart from these figures, the site of the tumors arising after the injection of filtrate free of foreign particles demonstrates the importance of cell-derangement. In three cases the growth had its primary seat in a functioning ovary, where injury and proliferation are of daily occurrence. In the fourth the growth was primary in the liver. Because of the general results, we feel that some focal derangement must have been present in this organ.

The factor of injury will not suffice of itself to explain the sarcoma's striking lack of infectivity under ordinary circumstances. During the past three years we have kept at one time or another in relatively close quarters, over 1,200 chickens, many of them with the sarcoma. To some the fresh, sarcomatous tissue has been fed, and many must have been contaminated with the dried tissue, in which, as we have found, the causative agent will remain active for over seven months. Trauma and other types of injury have been frequent among these chickens, yet not one has developed the sarcoma except when directly inoculated. Furthermore, judging from our recent examination of many "spontaneous" tumors the growth is not naturally endemic among fowls.

The nature of the other factors conditioning the tumor's origin has not yet been determined. It is evident that they are both local and general. Even with the agent in its present active form, and with the factor of injury supplied, many of the fowls injected with a large amount of filtrate fail to develop a growth. When the sarcoma follows an intravenous injection it is seldom primarily multiple, despite the numerous injuries everywhere caused by the infusorial earth. To reach the wing vein for injection we make an incision, thus producing an injury in which the tumor-producing agent might well be expected to localize; yet if the injecting needle is flushed with salt solution before being withdrawn, and

the vein is tied off, the occurrence of a growth at this site is practically ruled out. Our experiments to determine the precise stage of tissue injury or proliferation which favors the occurrence of a tumor have not given clear-cut results.

The conditions determining the incidence of the chicken sarcoma have considerable interest in view of its close resemblance to some malignant mammalian tumors, not only as regards growth and general behavior, but also in its obvious lack of infectivity under ordinary circumstances. This last feature is accounted for in the case of the chicken tumor by the factors on which depends the action of the growth's filterable cause. That injury should be a determining factor is noteworthy in view of its importance as a contributory cause of mammalian sarcomas, including those of human beings.

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THE RELATION BETWEEN A CHICKEN SARCOMA'S BEHAVIOR AND THE GROWTH'S FILTERABLE CAUSE *

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In several previous papers from this laboratory the behavior of a chicken sarcoma transmitted by a filterable agent has been described.[1] Emphasis has been laid on the growth's typically neoplastic character, and on the lack of phenomena suggesting the presence of an extrinsic causative agent. The importance of the subject in its possible bearing on the etiology of mammalian growths has led us to a further experimental study, here to be briefly reported.

An obvious point at which to attack the problem of the relationship between the avian growth and its cause is furnished by the process of metastasis formation. The histologic findings indicate more clearly than in the case of most tumors[2] the development of secondaries of the sarcoma from transported cells. But is this the usual method of the growth's dissemination? And, if so, are not at least some of the metastases due to a localization of the causative agent as such?

To test whether the sarcoma cells, as emboli, can proliferate and give rise to tumors has not been difficult. A suspension in Ringer's solution of particles of the fresh neoplastic tissue was injected intravenously into a number of susceptible, normal fowls, and at short intervals these fowls were killed and portions of the lungs examined in serial section. The mode of formation of the lung-tumors was in this way traced. It was found

* From the Laboratories of the Rockefeller Institute for Medical Research.

1. For the literature see an article by the authors, THE JOURNAL A. M. A., June 1, 1912, p. 1682.
2. Rous, Peyton: Jour. Exper. Med., 1911, xiii, 397.

that they uniformly arose by a survival and growth of the injected tumor cells, with penetration of the vessel-wall and extension into the surrounding tissue. The histologic pictures strikingly illustrate the development of a tumor by the multiplication of cells already neoplastic.

The visceral metastases of the sarcoma, occurring in the ordinary course of the disease, appear first and most frequently in the lungs. This rule is subject to exception much more rarely than in the case of human tumors metastasizing by the blood-stream. In only five of 157 fowls with visceral metastases were the lungs free of sarcoma and the further organs affected. In these cases, unfortunately, a patent foramen ovale was not looked for; but in two similar ones occurring since these statistics were compiled, the abnormality was present. Forty-one of the 157 fowls had metastases in the lungs alone; in 111 the lungs and other viscera were affected, often with very numerous secondary growths. In connection with the foregoing experiments, such a distribution is strong evidence for a general origin of the metastases from cell-emboli, which latter, as usual, are sieved out of the circulation by the pulmonary capillaries. There is, however, the alternate possibility that the extrinsic agent, as such, engenders tumors more easily in the lungs than in other organs. That this is not the case is shown by the results of direct intravenous injection of a Berkefeld filtrate containing the causative agent. In only two of eleven fowls developing a growth after such treatment was the sarcoma primary in the lungs, despite the fact that the agent was carried in bulk directly to these organs. In seven of the cases diatomaceous earth had been added to the filtrate and had lodged for the most part in the pulmonary capillaries, there producing injury such as has been found to favor the agent's action.[3]

Although it is thus certain that the chicken sarcoma's metastases are in general referable to a development of cell emboli, there remains the question whether secondary tumors may not be caused occasionally by the filterable agent as such. That the agent can enter the circulation is demonstrated by the appearance in some instances of a sarcoma after the injection into susceptible hosts of large quantities of centrifugated, "paraffin" plasma from

3. The Journal A. M. A., June 8, 1912, p. 1751.

fowls moribund with the metastasizing growth. The general importance of injury in determining the agent's action led us to attempt to obtain by its means secondary tumors under conditions which would show them to be independent in origin of transported cells.

Tissue derangements in tumor-bearing fowls were produced by the injection of scarlet red, or diatomaceous earth, or by incisions allowed to heal by secondary intention. The numerous experiments have given results almost entirely negative. Usually the sarcoma failed to localize at the seat of injury. A single case suggests the direct action of the extrinsic agent. In a chicken with a very large primary sarcoma, but no discoverable metastases in the viscera, a small secondary growth was found post mortem at the site of injection of diatomaceous earth. The heart was not examined for a foramen ovale. Two other instances of localization at the point of injury are less significant because of the presence of many metastases in the lungs and elsewhere. A third and similar case has come to attention during the routine autopsies on several hundred tumor-bearing fowls. The chicken had a large primary growth at the site of inoculation in the pectoral muscles, many metastases in the lungs but none in any other organ except the oviduct, in which were two small nodules. The oviduct contained an inspissated egg, and its walls showed reactive thickening and increased vascularization. Whether in any or all of these cases there was an action of the extrinsic agent, as such, cannot be said. When metastases develop at sites of injury in human beings the morphology of the growths often shows their origin from transported cells; but this feature is of no assistance with a spindle-celled sarcoma, such as ours, which might be primary anywhere.

The subsidiary part taken by the causative agent in the sarcoma's dissemination, if indeed it takes any direct part at all, is in keeping with what is already known of the tumor's growth and general behavior.[1] Yet in the end the sarcoma must be closely dependent on the activity of its filterable cause, for when this latter has been attenuated by a long stay in glycerin the tumors resulting from its inoculation develop slowly and frequently retrogress.

The conditions which determine the curious relationship between the disease and its cause are of much inter-

est. How does it happen that the sarcoma, though ultimately dependent on an extrinsic agent, is dominated in its behavior by the cells composing it? Some simple reasons suggest themselves. In the first place, the agent's action to produce a neoplastic change takes place with extreme slowness as compared with the proliferation of the cells, once they have become neoplastic. Consequently the growth of the neoplasm appears to take place entirely by multiplication of the cells of an initial focus. Growth doubtless does take place wholly in this way in many cases because of a second peculiarity of the agent, namely, its dependence on a special set of conditions in order that it may produce a neoplastic change.[3] The necessity for such conditions goes far to explain the agent's failure to take an active part in the tumor's dissemination in the body, granting indeed that the agent is present in the circulation before the fowl is moribund. The possibility of immune processes effective against the agent when separate from the cells must be kept in mind. The immune processes thus far recognized have been directed against the cells themselves, as in the case of the mammalian tumors.

The relationship existing between the chicken sarcoma and its cause, as shown by previous papers and by the findings here presented, seems to us to furnish some basis for the conception of an extrinsic cause for other sarcomata.

Sixty-Sixth Street and Avenue A.

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THE NATURE OF THE FILTERABLE AGENT CAUSING A SARCOMA OF THE FOWL*

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Although the filterable viruses have but recently come to attention, it is known that they are of very diverse character and that, except as a matter of expediency, they can scarcely be discussed together. At present each constitutes a separate problem. This is especially true of the filterable agent which causes a sarcoma of the fowl.[1] The disease is so different from the ordinary infectious processes that nothing can be preassumed with regard to the character of the etiologic agent. On the contrary, in considering it one must take into account all those numerous and often bizarre possibilities which are suggested by the literature on tumor-causation.

The first question concerning the agent is whether it is living. A very little of it will give rise to a growth from which numerous others may be started, each yielding the agent in abundance. But this does not necessarily settle the point. The agent might very well be a chemical substance somewhat similar to those used experimentally to produce atypical cell-proliferation. The fact that the proliferation induced by a chemical compound ceases as the latter is expanded, whereas our sarcoma's growth is continuous, constitutes no objection. For the sarcoma cells may themselves be the source of the inciting chemical, and thus serve to perpetuate the disease. Such an assumption throws little light on the origin of the "spontaneous" tumor which by transplantation has yielded our material.

The most direct means of proving that the agent is alive is to grow and transfer it in culture. Recently we

* From the Laboratories of the Rockefeller Institute for Medical Research.

1. Rous, Peyton: The Journal A. M. A., Jan. 21, 1911, p. 198.

have done much work in this direction without success. The results of differential filtration may have some value as indicating whether the agent is a formed body and its probable size. Here our recent results confirm those first reported. In a dilute tumor extract the agent will pass through Berkefeld filters which hold back at the same filtration *Bacillus fluorescens liquefaciens,* an organism measuring 0.5 micron by 1.0 to 1.5 microns. On the other hand, Chamberland bougies (F) prove impermeable despite conditions which seem very favorable to passage. Could the findings be accepted at their face value, they would go to show that the agent is organized and perhaps even visible. But many factors serve to invalidate negative results with filters of fine texture. As is well known, these may hold back the complex proteins as well as living organisms. Nevertheless, in view of the findings, we have made repeated attempts to demonstrate the agent with special stains or the dark-field microscope, but to no purpose.

The conditions under which the agent can survive have much interest. As elsewhere stated, it may retain its activity in dried tissue for seven months, and for at least one month in tissue placed in 50 per cent. glycerin. In both instances it undergoes a gradual attenuation, as evidenced by the behavior of the tumors it engenders. These appear in relatively few hosts and after a long interval; they grow slowly and retrogress frequently. The process of repeated rapid freezing and thawing, which reduces the tumor tissue to a pulp, does not markedly lessen the activity of the associated agent. The latter's resistance to heat, on the other hand, is little greater than that of the tumor-tissue itself, as has been shown by parallel inoculations of bits of the same heated material into susceptible fowls and the plasmatic medium of Burrows. Tumor-tissue submitted to a heat of 50 C. (122 F.) for fifteen minutes fails absolutely of growth in the plasmatic medium, in which its proliferation is ordinarily very active; but when inoculated into susceptible fowls it often gives rise to tumors and may do so even when it has been heated at 53 C. (127.4 F.). Whether growth in the plasmatic medium is a real index of the tumor tissue's viability is uncertain. But that the filterable agent will withstand a temperature of 50 C. for fifteen minutes is shown by the production of tumors with tissue which has been dried, ground and suspended in Ringer's solution previous to heating.

Material which has been heated to 55 C. (131 F.) for fifteen minutes never gives rise to the sarcoma.

In sarcomatous tissue autolyzing at the temperature of the chicken's body (41 C., 105.8 F.) the agent remains active for less than forty-eight hours. Toluol and chloroform in the proportions employed to prevent bacterial growth during autolysis destroy it in less than two hours. So, too, will 50 per cent. alcohol or 2 per cent. phenol (carbolic acid). Unlike the virus of poliomyelitis, the agent fails to withstand phenol 0.5 per cent. Like the animal organisms in distinction from most of the vegetable ones (von Prowacek) it is rapidly destroyed by bile, and by saponin in high dilutions. At 41 C. it is within two hours deprived of all activity by 50 per cent. rabbit bile or chicken bile, or by saponin in strengths greater than 1 to 800.

To obtain the foregoing data the only test employed, or at this time possible to employ in demonstrating the agent's activity, has been the inoculation of susceptible fowls. No single attribute among those determined suffices to show the nature of the agent; yet, taken together, its characters are those which we associate with microorganisms.

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A FILTERABLE AGENT THE CAUSE OF A SECOND CHICKEN-TUMOR, AN OSTEO-CHONDROSARCOMA *

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The demonstration of the cause of a transplantable spindle-celled chicken-sarcoma[1] led us last winter to a further study of tumors of the fowl. Spontaneous chicken-tumors are far from rare, and several excellent descriptions of them exist.[2] In the course of ten months we obtained without difficulty about thirty various tumors in the living fowl. None of the growths was quite similar histologically to the sarcoma just mentioned, which is caused by a filterable agent, but there were two that, like it, proved transplantable,[3] namely, a tumor producing true cartilage and bone (Chicken-Tumor 7) and a curious spindle-celled sarcoma (Chicken-Tumor 18). We wish here to report experiments showing that the osteochondromatous tumor is caused by a filterable agent.

The original osteochondroma appeared as a discrete mass on the breast-bone of an adult fowl. It was very hard, appeared to be of long standing, and projected symmetrically to either side of the sternal keel. Microscopically as well as clinically it seemed benign. That it grew on transplantation was surprising, but the less so since several mammalian growths of character apparently benign have proved transplantable, notably a chondroma of the mouse (Ehrlich) and a fibroma of the dog (Ribbert). Murray[4] has described a "chondro-

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* From the Laboratories of the Rockefeller Institute for Medical Research, New York.

1. Rous, Peyton: The Journal A. M. A., Jan. 21, 1911, p. 198.
2. Ehrenreich and Michaelis, Tyzzer, Wernicke.
3. Rous, Murphy and Tytler: The Journal A. M. A., June 1, 1912, p. 1682.
4. Murray, J. A.: Third Scientific Report of the Imperial Cancer Research Fund, 1908, p. 78.

osteoidsarcoma" of the mouse which retained on transplantation its ability to produce bone and cartilage. In the case of the chicken-tumor the growths derived from transplantation consist, like the original tumor, of large cells of connective tissue about which cartilage is soon laid down, followed, in many instances, by true bone containing red marrow. The neoplasm is now in its eighth transplantation generation. In some instances its growth is continuous, though often very slow, and as the mass of cartilage and bone becomes large the host emaciates and finally dies. In other instances the tumor sooner or later becomes stationary and may so remain for months. Recently metastases showing cartilage have been noted in the case of two of the most rapidly growing tumors. There is now prominent in the growth a spindle-celled element and it must be regarded as an osteochondrosarcoma. The general characters of the tumor are described in full in a paper by Dr. Tytler forthcoming in the *Journal of Experimental Medicine.*

Our attempts to determine a cause for the osteochondrosarcoma were not begun until after it had been several months under observation, and in numerous hosts. During this time its general character had altered little. The sarcomatous element was not noticeable and the growth appeared benign. Differential filtration was the method used. Sound portions of the tumor tissue were ground with sterile sand and taken up in a considerable bulk of Ringer's solution; shaking was done in a machine for from twenty to thirty minutes; centrifugalization followed; and then the supernatant fluid was passed through a filter of fine texture (Berkefeld) that held back all tumor-cells. A few cubic centimeters of the clear filtrate were injected into the trunk or leg muscles of a number of normal fowls. Sterile infusorial earth was in many instances added to the filtrate previous to its injection, since the tissue derangement caused by this foreign body has been found to favor the action of the agent engendering Chicken-Tumor 1,[5] the spindle-celled sarcoma.

For a first experiment a large Berkefeld filter (No. 8, V) was employed, without bacterial control as to its permeability. Of the ten fowls inoculated with 10 c.c. of the filtrate in each breast, one had developed thirty-eight days later a mass 0.3 cm. in diameter in the

5. Rous, Murphy and Tytler: The Journal A. M. A., June 8, 1912, p. 1751.

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muscle at one of the inoculation sites. At the end of another month, when the fowl died of intercurrent causes, this nodule measured 0.5 cm. It was very hard and consisted of the characteristic, cartilaginous tumor tissue, which, in this instance, contained no bone. At the present date, four months after the injection, the remaining fowls have not developed tumors.

In the later work Berkefeld filters of demonstrated impermeability to *Bacillus fluorescens liquefaciens* have been used. The development of malignancy in the chondroma, as shown by the production of metastases, has furnished a material which *a priori* would seem more suitable for the work. That it is indeed more suitable has been proved by the prompt development of swiftly growing tumors after the injection of the filtrate. For the experiment that follows there was taken the metastatic ovarian tumor of a fowl which carried in both pectoral regions large bony tumors as the result of implantation, and had in the ovary two hemorrhagic, cartilaginous metastases, each nearly as large as a hen's egg, together with diffuse implantation growths of the cartilage-forming connective tissue over visceral and parietal peritoneum.

EXPERIMENT

Cartilaginous tumor tissue to the amount of 11 gm. from the ovarian metastases of Fowl 352, sixth Generation A, was ground in a mortar with sterile sand. The resulting mass was taken up in about 350 c.c. of Ringer's solution warmed to body heat, and the whole was shaken in a machine for twenty minutes and briefly centrifugalized to sediment the tissue fragments. The cloudy supernatant fluid was passed through a Berkefeld filter V, size No. 3, which was tested both before and after the experiment under conditions similar to those obtaining in it, and was found to hold back *B. fluorescens liquefaciens* out of a suspension obtained by shaking. The tumor extract passed readily through the filter. The fluid thus procured was limpid and reddish-yellow (owing to hemorrhages into the cartilage).

Two portions of the filtrate, one with, and one without a little sterile infusorial earth *(Kieselguhr)*, were injected into each of six normal fowls of the breed in which the spontaneous tumor was found (Plymouth Rock). Of the filtrate plus *Kieselguhr* 4 c.c. were used for each injection; of the filtrate alone 6 c.c. The pectoral and thigh muscles were employed as sites.

Eighteen days later the first examination was made. In four of the fowls tumors were found where filtrate plus *Kieselguhr* had been injected. The growths measured from 1 to 3 cm.

across and were evidently due to the coalescence of numerous smaller nodules. In two of the four fowls a small, discrete nodule was palpable where the filtrate alone had been injected. It lay in the track of the injecting needle. All the growths were very hard. One of these fowls was at once killed and the growth looked at. Macroscopically cartilage could be seen in it. Microscopically it consisted of cartilage and a tissue, composed of numerous spindle-shaped or stellate cells, which was undergoing a cartilaginous change.

Twenty-five days after the injection the tumors were even harder than before and were growing rapidly. One now measured 4.7 cm. across. In two of the fowls the findings were still negative.

SIGNIFICANCE OF THE FINDINGS

The experiments show that the osteochondrosarcoma, like our spindle-celled sarcoma, can be produced in fowls hitherto normal, by an agent separable from the tumor cells and capable of passing through a Berkefeld filter which holds back *B. fluorescens liquefaciens.* Furthermore, the action of the agent appears to be dependent, like that producing the sarcoma, on a cell-derangement brought about by accessory factors. The addition to the filtrate of sterile infusorial earth to bring about a reactive tissue proliferation resulted in a high percentage of successful inoculations, each tumor arising from numerous local points; whereas the filtrate when injected alone in the same fowls caused a tumor much less frequently and then as a single discrete nodule in the track of injury from the injecting needle.

Despite these points of similarity the agents causing the chicken sarcoma and chondroma can hardly be identical. They give rise to widely different tumor forms. Previous to the experiments here related we had supposed it possible that a single filterable agent by its localization in cells of different potentiality might give rise to different tumors; to test the point we had made numerous attempts to obtain a localization of the agent causing Chicken-Tumor 1 in other cells than those which it usually influences. These attempts were all unsuccessful. And now it appears that the histologic character of the osteochondrosarcoma is due to a peculiarity of the causative agent which is retained when the latter is separated from the tissue of the growth. Thus the agent, when brought into contact with the connective tissue in voluntary muscle, produces not an ordinary spindle-celled sarcoma, but a growth that elaborates cartilage and finally bone. That an extra-

cellular agent should cause a reversion of connective tissue to the so-called embryonic type would not appear strange from what is already known of the changes that connective tissue undergoes in wounds, or when cultivated *in vitro;* but that such an agent should bring about a differentiation ordinarily foreign to the tissue is very remarkable. Yet in this connection it should not be forgotten that bone and, much more rarely, cartilage are sometimes laid down in connective tissue under relatively simple pathologic conditions.

The nature of the agent causing the osteochondrosarcoma cannot at present be stated. The agent causing the spindle-celled sarcoma is probably a living virus.[5] The demonstration that extrinsic agents are the cause of two connective-tissue growths of the fowl which are characteristic malignant tumors renders it necessary to suppose either that such tumors of the fowl have an entirely different etiology from mammalian tumors, or else that the latter are of similar origin. In any case the findings with the chicken tumors largely demolish the theoretical basis on which objections to an extrinsic cause for cancer have been built up.

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A TRANSPLANTABLE NEW GROWTH OF THE FOWL, PRODUCING CARTILAGE AND BONE.*

BY W. H. TYTLER, M.B.

(*From the Laboratories of The Rockefeller Institute for Medical Research, New York.*)

PLATES 79 TO 83.

Neoplasms of the common fowl have been described by numerous authors, and include tumors of various classes. Up to the present time four transplantable tumors of the fowl have been reported, one of which is the growth here dealt with. Fujinimi and Inamato in 1910 reported at a meeting in Japan a myxosarcoma which they had succeeded in transferring to other fowls.[1] In the same year Rous[2] described the transplantation of a spindle-celled sarcoma which at times showed myxomatous tendencies. This tumor was highly malignant, usually leading to the death of the animal, and often producing widespread metastases. The tumor reported by Fujinimi and Inamato appears to have been not so malignant, though sometimes metastasizing and causing death. The tumor which forms the subject of the present paper is an osteochondrosarcoma and has been mentioned in an earlier note[3] from this laboratory, in which was also reported the fourth transplantable chicken tumor, a spindle-celled sarcoma. For purposes of reference the osteochondrosarcoma is known in this laboratory as Chicken Tumor VII. It has great interest from at least two points of view. Its cells regularly undergo a process of metaplasia and differentiation to form cartilage and bone; and the growth is transmissible, like the spindle-celled sarcoma of Rous, by an agent separable from the tumor tissue and filterable through Berkefeld filters.[4] The filtrate,

* Received for publication, October 26, 1912.

[1] Fujinimi, A., and Inamato, K., *Verhandl. d. Jap. path. Gesellsch.*, 1911, 114.

[2] Rous, P., *Jour. Exper. Med.*, 1910, xii, 696; 1911, xiii, 397.

[3] Rous, P., Murphy, J. B., and Tytler, W. H., *Jour. Am. Med. Assn.*, 1912, lviii, 1682.

[4] Rous, P., Murphy, J. B., and Tytler, W. H., *Jour. Am. Med. Assn.*, 1912, lix, 1793.

when brought into contact with connective tissue under suitable circumstances, gives rise to the characteristic growth in which is laid down cartilage, followed often by bone.

DESCRIPTION OF THE ORIGINAL GROWTH.

The fowl bearing the original growth was obtained while yet alive from a dealer in poultry. It was a Plymouth Rock hen, in good condition, of approximately pure breed, and apparently about a year old. On the lower portion of the keel of the sternum was an irregularly spherical mass, so symmetrically disposed that the keel passed almost through its center. It measured 6.6 by 5.7 by 4 centimeters. The growth was smooth, nearly as hard as bone, and the skin over it was slightly stretched but not firmly attached (figure 1). A wedge-shaped piece of it was taken out under ether anesthesia. The tissue thus obtained was found to be firm, white, and fibrous, with some translucent areas suggesting cartilage. Small bits were at once implanted in the original fowl, by means of a trocar (Bashford needle). Two such grafts were placed in the pectoral muscles, and four subcutaneously, on each side.

An examination of the fowl thirty-six days later showed that the original mass had not grown, except perhaps at the edges of the recent incision. The gap left by the removal of tissue had persisted almost unchanged, and was filled with purulent material. At six of the ten inoculation sites small nodules were felt. The fowl was in poor condition, so it was killed, and bits of the tumor tissue were inoculated into twenty young, pure-bred Plymouth Rock hens, the trocar again being used. Two of these died of intercurrent disease, but in twelve of the eighteen that survived growths developed, appearing in general within thirty days. The propagation of the tumor has since been easy, and it is now in its seventh transplantation generation.

GENERAL MORPHOLOGY OF THE GROWTH.

The general morphology of the transplantation tumors does not differ from that of the original or spontaneous growth. The latter was removed at autopsy together with the lower half of the

sternum, and was cut transversely It was well encapsulated. In the gross specimen the sternal keel could be traced to the growth's center, but here it was lost in a mass of red, spongy, bony tissue which radiated from it a distance of one to one and one half centimeters (figure 2). Peripheral to this the tumor was hard and white, with fine strands of opaque, fibrous tissue separating more translucent, homogeneous areas. The secondary nodules, globular and well defined, were composed of the opaque, fibrous tissue. The fowl was emaciated. No metastases were found.

Microscopically the capsule of the original growth was found to consist of fibrous connective tissue containing isolated muscle bundles. The growth itself was made up of a zone of what may be termed prechondral tissue, enclosing and grading into a mass of hyaline cartilage through which ran the sternal keel. From this latter numerous irregular, bony trabeculæ radiated into the cartilage.

HISTOLOGICAL FINDINGS.

The outer, or prechondral, tumor tissue is an irregular connective tissue composed of sparsely scattered cells in a relatively large amount of fibrillar intercellular substance. The cells closely resemble normal connective tissue cells of the young fibroblast type, having large vesicular nuclei, some with two nucleolar masses and some showing amitotic division or nuclear budding. The cells lie, singly or in groups, in slit-like spaces, and in the groups are larger and more globular. Blood vessels are numerous, and their walls consist usually of endothelium only. The intercellular substance gives the staining reaction for collagen.

Deeper in are occasional islands in which the cells are polyhedral or globular, and lie in irregular spaces suggesting capsules, while the intercellular substance is here relatively homogeneous, though non-staining. These islands strongly suggest cartilage, and in fact all transitions are found between them and actual cartilaginous areas lying further toward the center of the tumor. The cartilage cells are globular, in well formed capsules, and the ground substance stains deep blue with hematoxylin, taking an intense blue at the capsule rims. With aniline blue the ground substance takes a light, diffuse stain and also shows numerous fine, deeply staining, collagen fibrils.

Near the center of the growth the cartilage undergoes a transition to a non-calcified, osteoid tissue, which itself undergoes calcification and partial resorption. It is in this way that the radiating bony trabeculæ already mentioned appear to have been formed. The deeply staining cartilage masses, at their inner border, shade into a zone with a non-staining, homogeneous ground substance, the cartilage cells at the same time showing various stages of transition to cells with the general morphology of bone corpuscles (figure 7). Still further

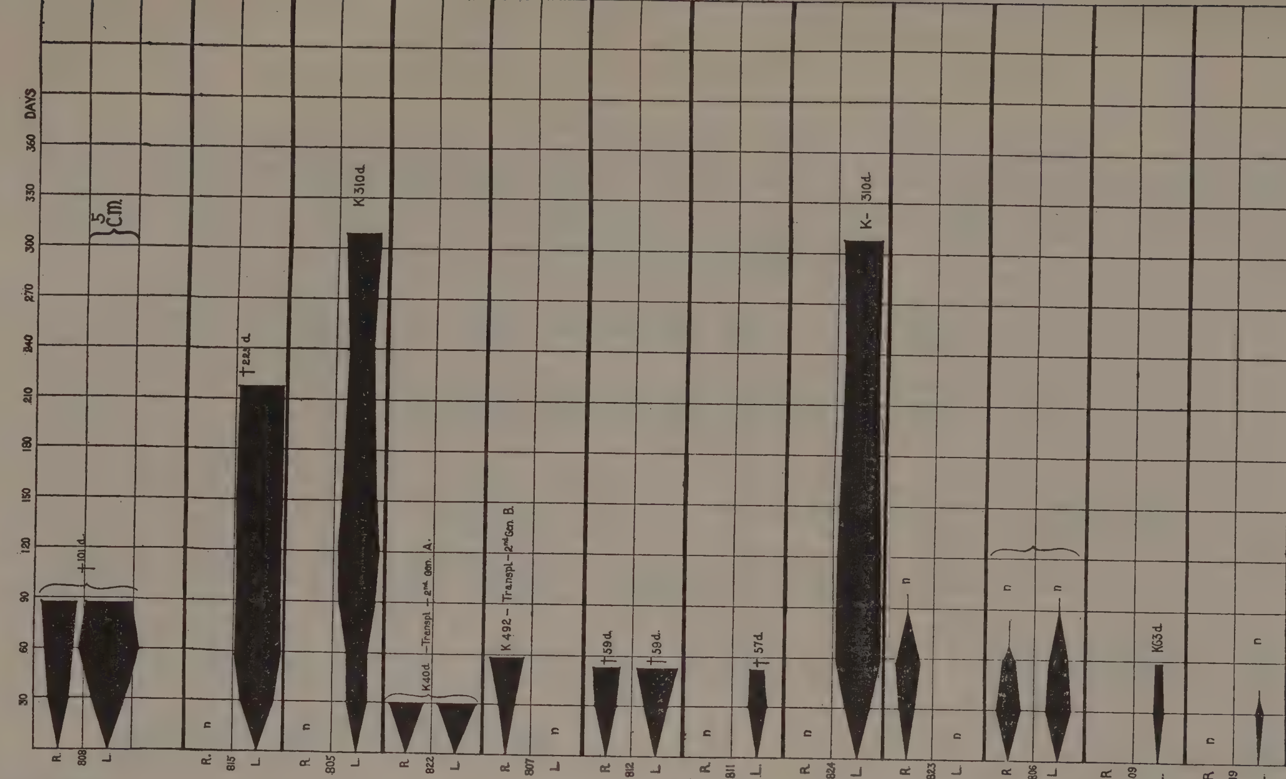
in, calcification is taking place with the formation of trellis-like figures, staining deep blue with hematoxylin, between the cells of the osteoid tissue. Into this calcified zone, which in most places is quite narrow, marrow spaces, with their blood vessels and osteoclasts, are advancing, and resorption is taking place. Thus are formed irregular bony trabeculæ. The intertrabecular marrow spaces contain a loose connective tissue, for the most part only sparsely cellular, but in places packed with cells that resemble fibroblasts. Occasionally small collections of granular leucocytes, mostly of the myelocyte type, are seen. Blood vessels are small and not numerous. Deep in the mass the keel of the sternum is recognizable as two roughly parallel bony plates (figure 4). The bony trabeculæ which radiate from the outer surface of these into the tumor tissue closely resemble the trabeculæ of the medullary cavity. The periosteum can be traced from the base of the sternum up to the lowest of these bony processes on each side, but here it is lost in tumor tissue. Toward its free edge the sternal keel is broken through in many places and here the newly formed bone is continuous with the medullary trabeculæ. The edges of the gaps show active erosion by osteoclasts.

The secondary subcutaneous and intramuscular nodules consist practically entirely of prechondral tissue of the sort described. This tissue is much more cellular than in the original tumor, the cells lying close together with but a small amount of intercellular substance. Many of them have two nucleoli, and some show amitotic division in progress, or, more rarely, a mitotic figure may be seen. At its margin the prechondral tissue extends out between the muscle bundles, while external to this there is, amid the muscle, a diffuse increase of connective tissue.

To recapitulate briefly: The outermost, youngest zone of the tumor is made up of cells of fibroblastic type scattered sparsely in a collagenous intercellular substance. In its deeper portions this tissue is undergoing a transformation to cartilage, its cells taking the character of cartilage cells, while the ground substance becomes homogeneous and basic staining. The original growth is well encapsulated, and seems nearly, if not quite, stationary. The secondary tumors have grown rapidly, are more cellular than the primary growth, and are invasive in tendency.

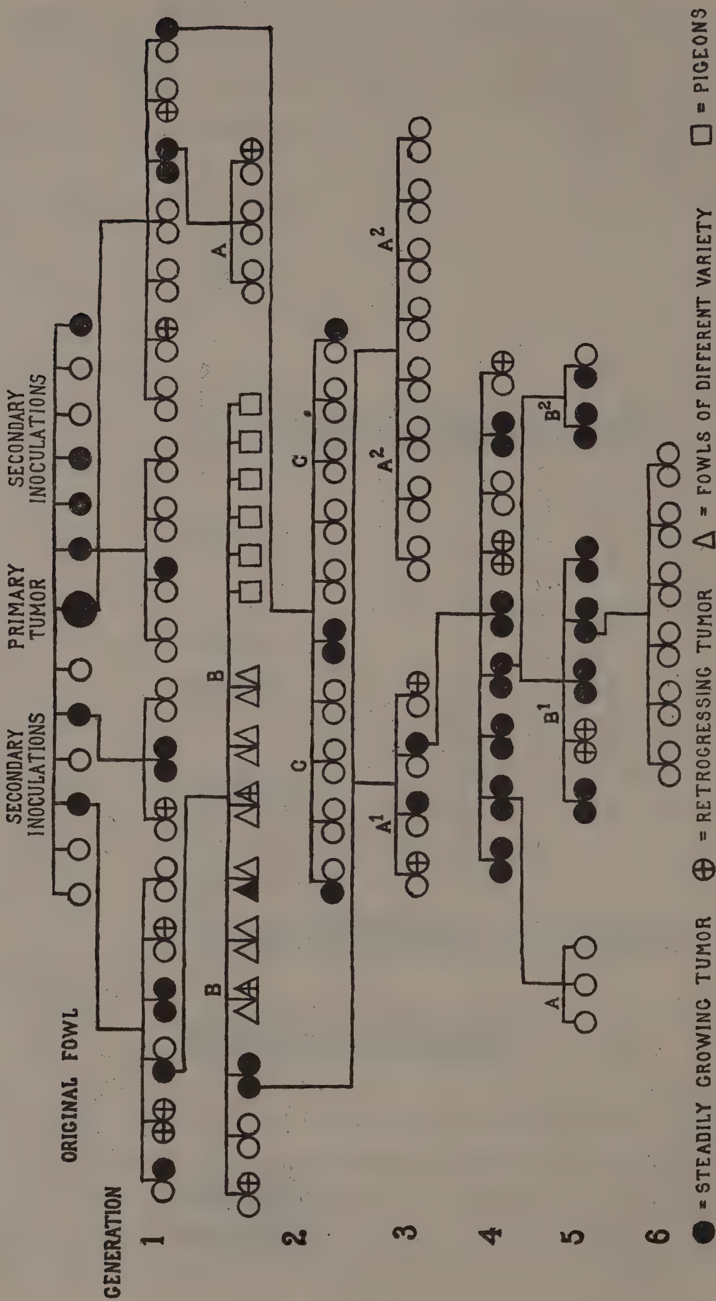
GENERAL RESULTS OF TRANSPLANTATION.

The growth has been propagated in series of chickens by transplanting small bits of the fresh, peripheral, neoplastic cartilage. In general, Plymouth Rock chickens have been used, and by means of a trocar the grafts have been placed deep in the pectoral muscles. In one series of four fowls prechondral tumor tissue was placed in one breast, in the other cartilage from the same tumor. Growths



TEXT-FIG. I. Chart showing the results of implantations in the right and left pectoral regions of the twelve susceptible fowls of the first transplantation generation. The tumor gro wth is represented as a black band, the thickness of the band being that of the average diameter of the growth at any one time, and its length that of the number of days that the growth endured. The angular outlines of the bands are referable to the fact that they were plotted from measurements taken at considerable intervals. Rapid growth followed by slowing or retrogression is characteristic of Chicken Tumor VII.

From the figure it would appear that the tumors began to grow immediately on the implantation of the grafts. This was probably not the case, but a more correct chart cannot be made since the first examination did not take place until thirty days after the implantation. 809, 810, etc. = index num ber of the individual fowl. *R* and *L* = right and left pectoral regions.



TEXT-FIG. 2. The fate of the osteochondrosarcoma in the first five tumor generations. One negative series of the sixth generation is also figured.

developed in all four hosts, but only from the cartilage. The results of the transplantation from the original fowl are shown in text-figure 1, which also illustrates well the general behavior of all the propagated growths. The varying success of the tumor in its first five transplantation generations is shown in text-figure 2.

The percentage of successful inoculations from the primary growth was very high,—60 per cent. of the fowls yielding tumors and growths developing at 40 per cent. of all the inoculation sites. The first transplantation of spontaneous mouse tumors does not ordinarily yield nearly so high a proportion of growths. It is interesting to note that in Haaland's[5] series of seventy-eight spontaneous mouse tumors that were transplanted, the greatest average success was obtained with growths that on transplantation showed a general tendency to retrogress. In the case of Chicken Tumor VII this tendency is very marked.

Passage from host to host has scarcely increased the transplantability of the chicken tumor (text-figure 2). Furthermore, at present (the seventh tumor generation) it grows little if any more rapidly than when first transferred. But the prechondral tissue frequently infiltrates the surrounding muscle; and in one recent case a metastasis was found. For these reasons the tumor is best considered as an osteochondrosarcoma.

The development of Chicken Tumor VII after the implantation of a graft follows certain regular lines. The immediate fate of the implanted tissue has not yet been traced, but where it is placed in susceptible fowls there appears after ten days or two weeks a discrete, smooth, firm nodule which usually enlarges rapidly. It is ellipsoidal with its long axis in the direction of the muscle fibers. In one instance a mass four centimeters in diameter developed within thirty days after the implantation of a tumor bit not larger than four by one millimeters. There is often some invasion of the tissue surrounding such a nodule, but its growth is predominantly expansive in character and often it can be shelled out of its capsule, which at this time is usually well developed. Microscopically the tumor is found to consist of the peculiar prechondral tissue that

[5] Haaland, M., *Fourth Scientific Report of the Imperial Cancer Research Fund,* 1911, 1.

has been described in connection with the original growth. Cartilage is already forming at its center, as islands which gradually enlarge and merge into a mass that seems homogeneous to the naked eye, and as the mass grows larger degenerative changes occur at its center. With the gradual transformation to cartilage there takes place a slowing of the tumor's growth rate (text-figure 1), and after about fifty days nearly all the tumors become stationary, and many begin to retrogress. In some of the growing tumors, the proliferation of the prechondral rim and its transformation to cartilage slowly continue. If the host lives long enough ossification occurs within the cartilage and nearly the whole tumor may be converted into bone. The bony change takes place quite independently of whether the tumor is growing, stationary, or retrogressing, but in any event bone does not appear in the growth until the third or fourth month.

Instances in which this tumor has led to the death of the host are as yet few. Even when quite large it does not appear to cause ill health. At present there are in the laboratory numerous fowls which for many months have carried the growth in the breast as a stationary, retrogressing, or slowly growing lump of bony hardness. In the instances in which the tumor proved fatal the host has gradually emaciated and at length become comatose for a day or so before death. At autopsy no changes were found in the viscera other than those attendant upon emaciation and secondary anemia.

SPECIMEN PROTOCOLS.

A Progressively Growing Tumor.—Fowl 504, 7th generation A. In each breast of this fowl there was implanted a graft from No. 321, 6th generation A. In the right breast there soon developed a growth like a slightly flattened sphere, lying within the muscle, which increased rapidly in size up to the time of the fowl's death, sixty-seven days after the implantation. At autopsy the mass measured 6.4 by 5.6 by 4.5 cm. It projected sharply from the breast of the emaciated host. It was not attached to the sternum, but the pressure of its enlargement had arched inward the membrane stretching between two sternal ribs lying directly behind it. The growth was very firm and on section discrete, grayish white, of smooth, close texture, and translucent. Its center was honeycombed with blood channels, and showed at one point the old clot of a hemorrhage, at another a few bony spicules. Microscopically the growth consisted of hyaline cartilage with a thin rim of prechondral tissue. The central blood channels were walled only by endothelium. No metastases were found anywhere. It is doubtful if death was due to the tumor.

A Metastasizing Tumor.—Fowl 46, 2d generation C. A bit of tumor from No. 824, 1st generation A, was implanted in the muscle of each pectoral region of this fowl. Three weeks later a nodule about 1 cm. in diameter had developed at one site and after two months a similar growth appeared at the other. The tumors grew slowly and at length became stationary, remaining so for many weeks before the fowl's death which occurred from intercurrent causes seven months after the inoculation. At autopsy the tumors were found to be shaped like slightly flattened spheres. They measured 5 and 1.7 cm. respectively, in their greatest diameter, were discrete and stony hard, and consisted of a narrow, gristly rim of cartilage about a mass of spongy bone and red bone marrow.

On cutting away the sternum a characteristic tumor mass, 0.9 cm. in diameter, was disclosed, projecting sharply into the thoracic cavity and attached by a narrow base to the inner surface of the lowest right rib at the junction of its sternal and vertebral portions. Its situation was such that it could not be attributed to the primary implantation. It contained cartilage.

A Stationary, Bony Tumor.—Fowl 823, 1st generation A, and 6th generation A. This fowl was inoculated with two grafts of the original osteochondrosarcoma, and developed as a result one small tumor which was largely removed at operation, the remainder of it retrogressing. One hundred and five days after its disappearance a reinoculation was done, this time with material from No. 353, 5th generation B. A tumor developed which within a month reached a diameter of 2.5 cm., but then ceased to grow. Four months later, when the fowl was killed, it measured only 2 cm. across. At autopsy it proved to be a roughly spherical, bony nodule, with a smooth surface and a thin connective tissue capsule. It was made up of an outer shell or lamella of bone enclosing spongy bone and red marrow and a few islands of cartilage. The general health of the host had been good throughout.

HISTOLOGY OF THE METAPLASIA.

The formation of cartilage which takes place regularly in the transplanted growths (figures 5, 9, and 10) comes about in the same manner as in the original tumor. The islands of cartilage, at first isolated in prechondral tissue, gradually enlarge and become more numerous until to the naked eye their fusion appears complete. As a matter of fact, the microscope shows that they are still separated by thin strands of the prechondral tissue. In its interior the cartilage sooner or later undergoes degeneration, with loss of staining power and gradual death of the cells.

The change to bone also occurs in the same way as in the primary tumor. The cartilage undergoes a transformation to osteoid tissue, becomes calcified, and is actively eroded by osteoclasts to form trabeculæ between which red bone marrow appears (figure 6). There is thus formed an irregular mass of spongy bone. The

process, as in the primary tumor, seems to be one of progressive resorption, with no production of new bone except as is obtained by the continuous conversion of cartilage into a calcified, osteoid tissue. Osteoblasts appear to play no part in the bone formation.

The bone cells are somewhat irregular in morphology, but the majority closely resemble normal bone corpuscles, being small, flattened, sharp angled, dark staining cells, lying in clear cut spaces. The ground substance is dense and homogeneous. The marrow spaces show large blood spaces, walled with endothelium and distended with red blood cells. Most of the red cells are not peculiar, but many are more globular than the normal and have a bluish grey cytoplasm (after eosin and methylene-blue) and pale, rounded, vesicular nuclei. Occasionally a mitotic figure is seen in one of these cells.

In the intervascular tissue are seen numerous spindle cells of the type seen at the growing border of the tumor. These cells occur also in more globular form, many with few or no cytoplasmic processes, but with the same large vesicular nuclei and pale staining cytoplasm as the prechondral cells. Small numbers of cells occur, however, of the same size and shape as these globular cells, and with the same type of nucleus, but with a strongly basophilic cytoplasm which may contain occasional eosinophilic granules. The nuclei occasionally show mitosis. These cells closely resemble the strongly basophilic cells seen in the development of human blood, and designated as *Wanderzellen,* myeloblasts, etc., by various authors (Maximoff, Askanazy, Schridde, Naegeli, and others). With these cells occur mononuclear cells filled with eosinophile granules, and the ordinary polymorphonuclear eosinophile leucocytes. These four types of cells, the globular, pale staining connective tissue cell, the basophilic cell with few or no granules, the myelocyte, and the polymorphonuclear leucocyte, are often seen lying together in nest-like collections, the last two types always being in excess. The suggestion is very strong that the myelocyte type takes its origin from the basophilic cell, which in turn seems to be related to the globular connective tissue cell. Whether these latter take their origin from tumor cells, representing thus another metaplastic change, has yet to be determined.

The striking characteristic of the retrogressing neoplasms is the replacement of tumor by fibrous connective tissue which from its maturity and uniformity would appear to be derived from the host tissues. The tumor is separated by this into small, sharply defined nodules, which consist of cartilage, or, in the case of the more outlying ones, of dense collagenous tissue that is still living. At the margin of the mass are many good sized blood vessels, surrounded usually by numerous lymphocytes. In old, stationary growths cartilage and prechondral tissue may practically disappear leaving a rounded, bony nodule consisting of a smooth outer lamella, or bony shell, and a spongy interior of trabeculæ and red marrow.

INTRAVENOUS INOCULATION.

Three fowls of the 5th generation A received at the time of their original intramuscular inoculation an intravenous injection of a suspension of the finely ground tumor in Ringer solution. All fowls developed tumors in the breast muscle as a result of the inoculation at that site. Two of them showed at autopsy no visceral lesions. In the third fowl, No. 352, dying 136 days after the injection, there were found, in addition to the large, bony, pectoral growths, two tumor masses attached to the ovary, and each the size of a hen's egg, in which coils of small bowel were involved. There were also irregular disseminations of tumor tissue, up to 0.5 cm. in thickness, on the parietal and visceral surfaces of the peritoneum. Microscopically both the ovarian and the peritoneal growths showed characteristic prechondral tissue and cartilage. The ovarian tumors also showed extensive hemorrhage and necrosis. Whether these visceral growths resulted from the material injected intravenously, or were secondary to the pectoral tumors, cannot be definitely said. The relatively slight metaplastic changes observed in them favors the latter conjecture since it points to a recent origin for them.

BACTERIOLOGICAL EXAMINATION.

Smears taken from the tumor tissue and stained by various methods have failed to reveal any structures suggesting living organisms, as has also examination with the dark-field microscope.

Cultures were made on all the usual laboratory media, both aerobically and anaerobically. No growth resulted, save on two tubes which showed evident contamination.

IMMUNITY.

Several experiments have been made in the attempt to demonstrate the occurrence of an immunity, natural or acquired, against the tumor. The results obtained up to the present hardly seem to justify definite conclusions, owing partly to the comparatively small number of animals tested, and partly to a lack of uniformity in the material used for reinoculation, as shown by the results in control fowls. The following is a summary of the findings.

In the 3d generation B there were inoculated three Plymouth Rock hens, three black Minorca hens, three brown Leghorn hens, and six mongrel pigeons. No tumor growth took place in any of the pigeons, suggesting, for that species at least, the occurrence of a natural specific immunity, as is the case with animal tumors in general. Tumors developed, however, in both of the alien breeds of the same species as the original host. A difference of breed does not, then, constitute a barrier to the transplantation of Chicken Tumor VII. In this respect the tumor contrasts sharply with Chicken Tumor I (Rous[6]), which in its earlier generations could be transplanted only to fowls of close blood relationship to the original host.

Reinoculations have been made in several series of animals, including fowls which remained negative from the first inoculation, fowls bearing growing or retrogressing tumors, and fowls in which the growth had retrogressed completely. Reinoculation of fowls negative from the original implantation gave tumors only in one series of three fowls. These had been inoculated originally with tumor emulsion, which gave completely negative results. The other fowls of this class, twenty-two in all, still showed no growth after the second inoculation. Although the value of the result was lessened, for a part of this series, by the unsuitable nature of the material used for reinoculation, the findings suggest the occurrence of a strong natural immunity in certain individuals of the species.

[6] Rous, P., *loc cit.*

In the case of fowls bearing tumors, or in which tumors had retrogressed, reinoculation gave a very small percentage of tumors as compared with the general results in normal fowls. The interpretation of the findings is complicated by several factors, which are important, one being the general poor health of fowls which have been confined for some time in close quarters. The inference is apparently justified, however, that a certain degree of resistance develops in animals in which the tumor has grown for any considerable period. If this is so it offers one explanation for the general tendency to retrogression which Chicken Tumor VII exhibits.

DISCUSSION.

The osteochondrosarcoma described occurred as a unique instance among some thirty primary chicken tumors brought to this laboratory during the last twelve months. It appears to have arisen from the periosteum, all the layers participating in the growth. Injury must be thought of as a contributory cause, because of the tumor's position. Its symmetrical arrangement and histological structure at first suggested that it was congenital in origin, but no evidence confirmatory to this view has been obtained.

In its general characters Chicken Tumor VII is like certain mouse tumors, also osteochondrosarcomata, described by Haaland[7] and Murray.[8] Murray's tumor was successfully transplanted and retained its ability to produce cartilage and bone. From his description it appears that these tissues were laid down in a spindle-celled parenchyma, much as happens in the case of Chicken Tumor VII. It is interesting in this connection to remember that dogs are not infrequently the subject of mixed tumors of the mamma which contain an epithelial element, cartilage, and bone, and occasionally give metastases showing one or more of these tissues. The development and differentiation which takes place in Chicken Tumor VII is somewhat similar to that occurring in the normal growth of the chick embryo, but in other ways is notably different. The tumor cells in their early form are relatively simple looking con-

[7] Haaland, M., *Ztschr. f. Krebsforsch.*, 1907, v, 125.

[8] Murray, J. A., *Third Scientific Report of the Imperial Cancer Research Fund*, 1908, 69.

nective tissue cells, and they become changed into elements which, save for the fact that they are hypertrophic, are wanting in none of the morphological characters of normal cartilage cells. At the same time an intercellular substance is formed which shows the morphological features and the staining reactions of the ground substance of normal cartilage. But between the connective tissue stage and the cartilage stage is an intermediate one during which the cell produces a large amount of collagenous material. Such a stage is not prominent in the development of the normal cartilage of the bird.

In many instances of Chicken Tumor VII the cartilage at length undergoes degeneration and death, possibly from insufficient blood supply. In others, or in other parts of the mass the cartilage is changed to an osteoid tissue which later undergoes calcification and erosion by osteoclasts, with resulting formation of bony trabeculæ. The bone formation takes place, as a rule, only in connection with the marginal healthy cartilage, so that a peripheral bony zone may be found enclosing a soft degenerated center (figure 3).

The process of bone formation differs in its order from that of normal endochondral bone in the chick, as described by Lillie,[9] for in the production of normal bone the cartilage is first calcified and then eroded, while at the same time osteoid tissue is deposited on the trabeculæ by osteoblasts. Whether in Chicken Tumor VII the bone marrow develops from neoplastic tissue or by an ingrowth of tissue elements from the host is unsettled, but the former process is suggested by the findings as an origin for many of the marrow elements, as is also the possibility of even certain of the blood cells having arisen from tumor tissue. Both hypotheses, of course, require more extensive confirmation.

SUMMARY.

An osteochondrosarcoma of the common fowl, designated in this laboratory as Chicken Tumor VII, has been successfully transplanted to seven successive series of hosts. The original growth contained bone and cartilage, was attached to the sternal keel of an otherwise healthy chicken, and appeared to have arisen from this

[9] Lillie, F. R., The Development of the Chick, New York, 1908, 409.

structure. In the growths derived from its transplantation cartilage is regularly laid down, followed later by bone in case the host lives long enough. The prechondral tissue consists of spindle-shaped or multipolar cells of the fibroblast type. The histological character and the behavior of this prechondral tissue show that it is sarcomatous, a fact further proven by one recent case in which the tumor has metastasized. The secondary growth in this instance consisted of prechondral tissue in which a cartilaginous change was taking place.

The tumor could not be transferred to pigeons, the one foreign species tested, but grew readily in chickens of two alien breeds. Reinoculation experiments suggest the occurrence of a natural, individual immunity, and of a certain degree of acquired resistance. In one fowl visceral growths developed following an intravenous injection of tumor emulsion, although whether they were due to this cause or were secondary to the large implantation growths in the muscles is uncertain. Recently the tumor has been transmitted by means of the filtrate from a Berkefeld filter.

EXPLANATION OF PLATES.

Plate 79.

Fig. 1. Original tumor with a part of the sternal keel, viewed from the right side. The skin has been stripped away except over the crest of the keel. About three fourths natural size.

Fig. 2. Section through the center of the tumor and across the keel of the sternum. The keel lies vertically in the midst of the growth but is lost in a mass of spongy bone. A wedge-shaped piece of tissue has been cut from one side of the tumor. The dark areas deep in the growth are areas of spongy bone. $n =$ the notch left by excision of the tissue; k-$k =$ the position of the keel; $st =$ the sternum at the base of the keel. About natural size.

Plate 80.

Fig. 3. Cross section of a bony tumor resulting from transplantation. The growth was eighty-five days old and had been stationary for some weeks before its removal. In this instance a broad zone of spongy bony tissue surrounds a degenerated center. The growth is well encapsulated. About natural size.

Fig. 4. Original tumor. Microscopic preparations showing the disappearance of the sternal keel amid the growth's bony trabeculæ. At the upper left hand corner of the photograph is a dark mass of cartilage, which is separated from the bone by a layer of osteoid tissue nearly free from stain. k-$k =$ lamellæ of the keel. Stained with hematoxylin.

PLATE 81.

FIG. 5. Transplantation tumor (fowl 822, 1st generation). Duration of growth, thirty-five days. Microscopic preparation showing prechondral tissue and its transformation into cartilage. Stained with eosin and methylene-blue.

FIG. 6. Transplantation tumor (fowl 808, 1st generation), showing bony trabeculæ, osteoclasts, and marrow with myelocytes. Stained with hematoxylin.

PLATE 82.

FIG. 7. Original tumor. At the right of the picture there is cartilage, next to this osteoid tissue (nearly unstained), and to the left calcification of the osteoid tissue with marrow spaces advancing into the latter. Stained with hematoxylin.

FIG. 8. Hypertrophic cells of hyaline cartilage from a transplantation tumor (fowl 806, 1st generation). One cell has three nuclei. Stained with Mallory's chloride of iron hematoxylin.

PLATE 83.

FIG. 9. From a transplantation tumor (fowl 815, 1st generation), showing transformation of collagenous connective tissue to cartilag . Note the large blood sinuses. Stained with hematoxylin.

FIG. 10. Another portion of the same growth, more highly magnified, showing collagenous neoplastic tissue (prechondral tissue) with cells resembling fibroblasts. Part of a blood sinus walled by endothelial cells is also shown. Stained with Mallory's chloride of iron hematoxylin.

FIG. 1.

FIG. 2.

(Tytler: A Transplantable New Growth of the Fowl.)

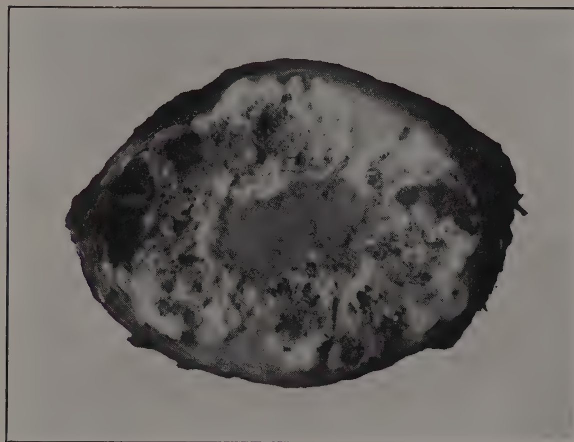


Fig. 3.

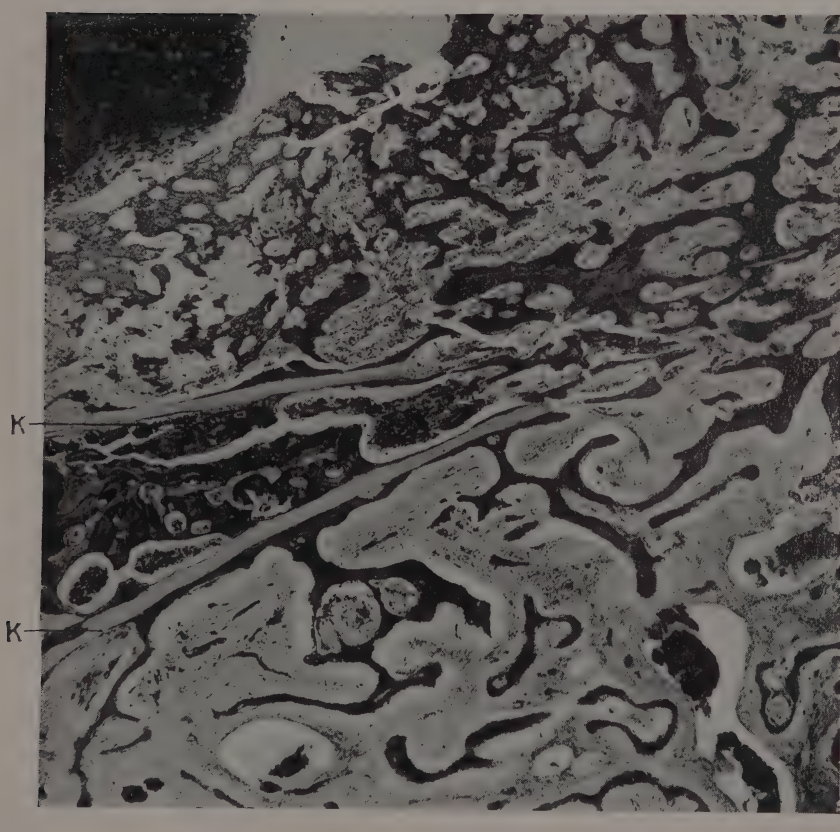


Fig. 4.

(Tytler: A Transplantable New Growth of the Fowl.)

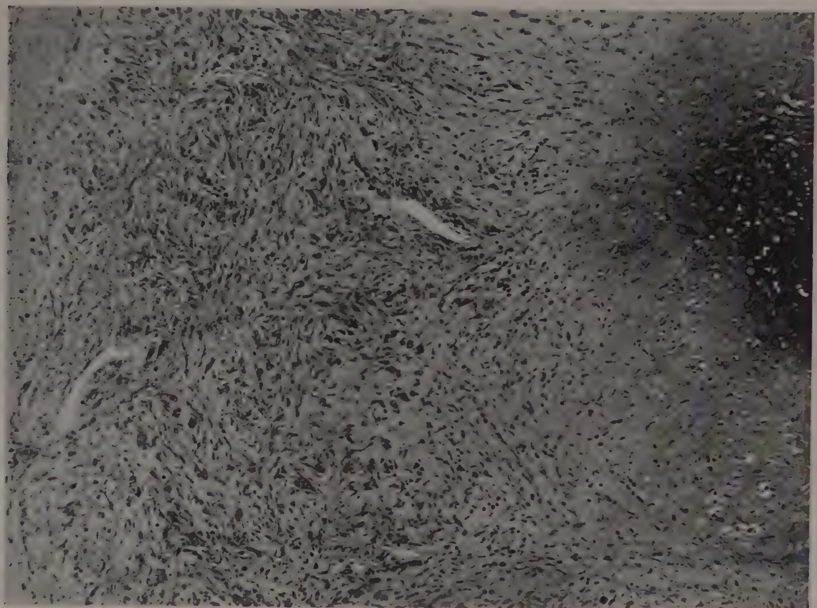


FIG. 5.

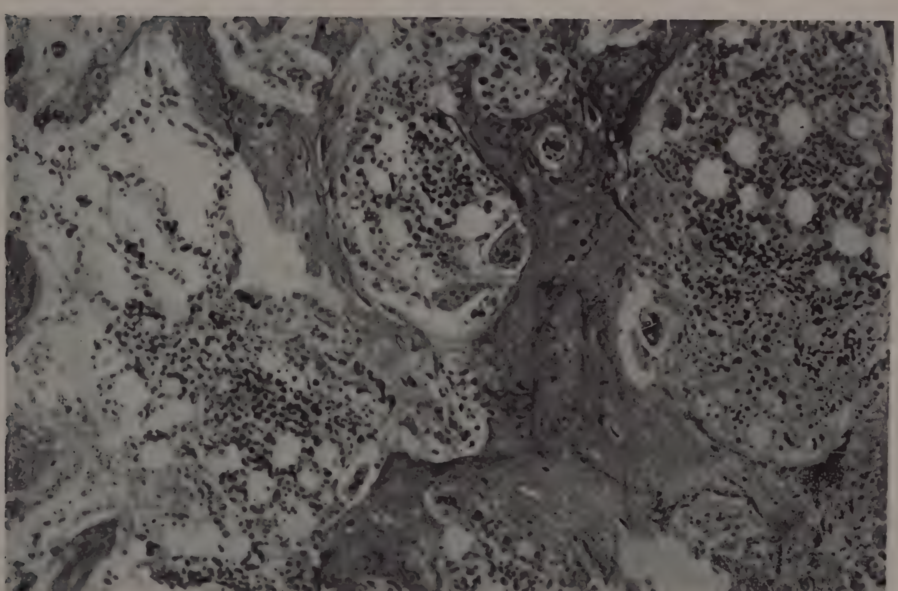


FIG. 6.

(Tytler: A Transplantable New Growth of the Fowl.)

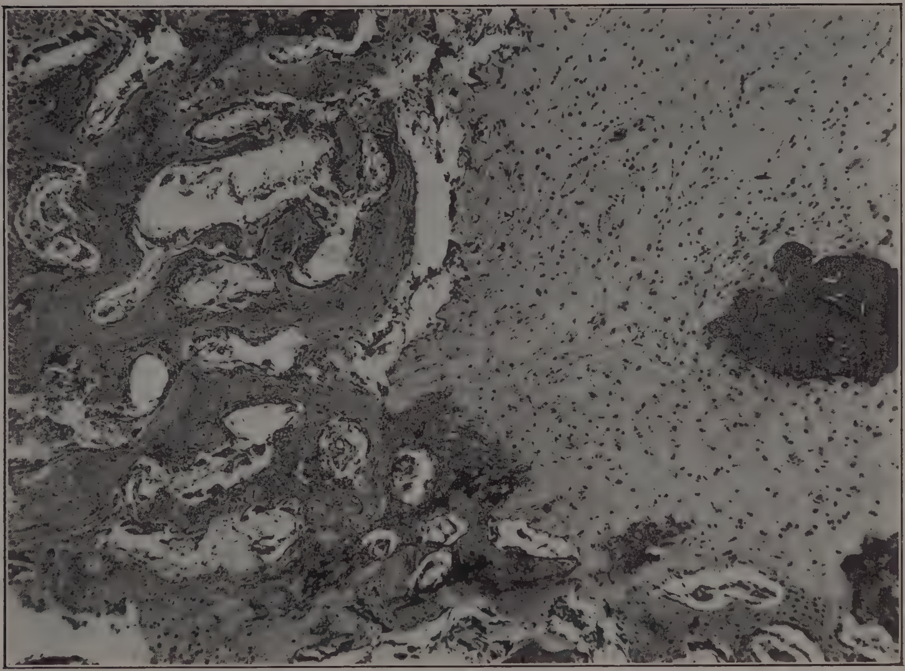


Fig. 7.

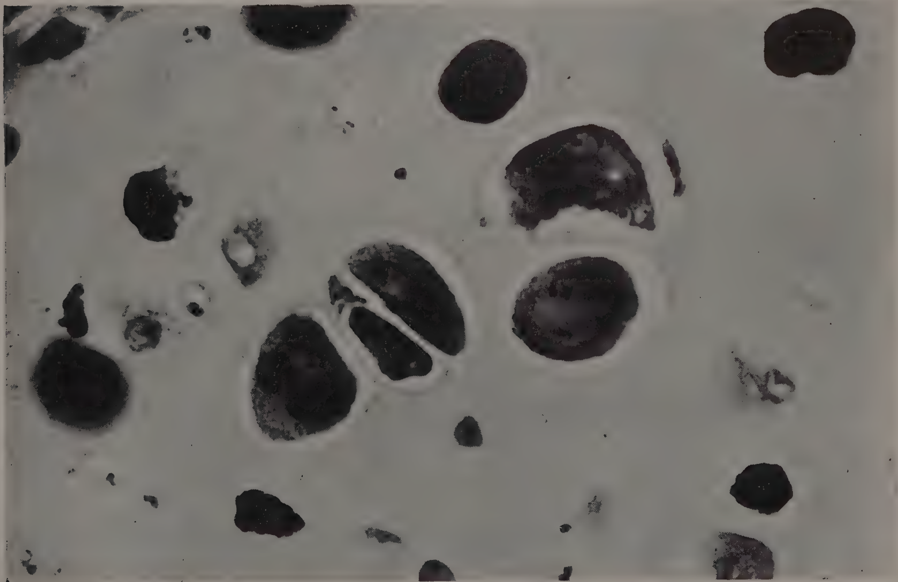


Fig. 8.

(Tytler: A Transplantable New Growth of the Fowl.)

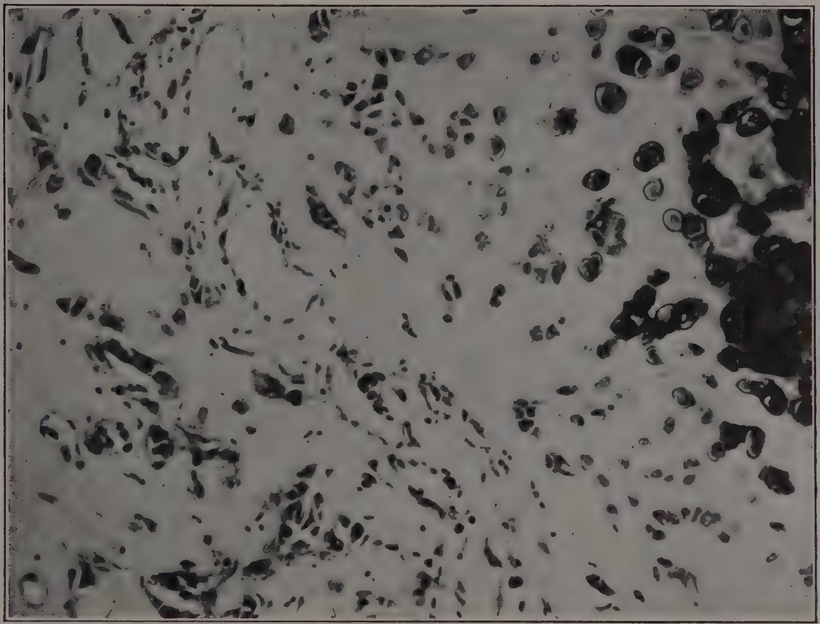


FIG. 9.

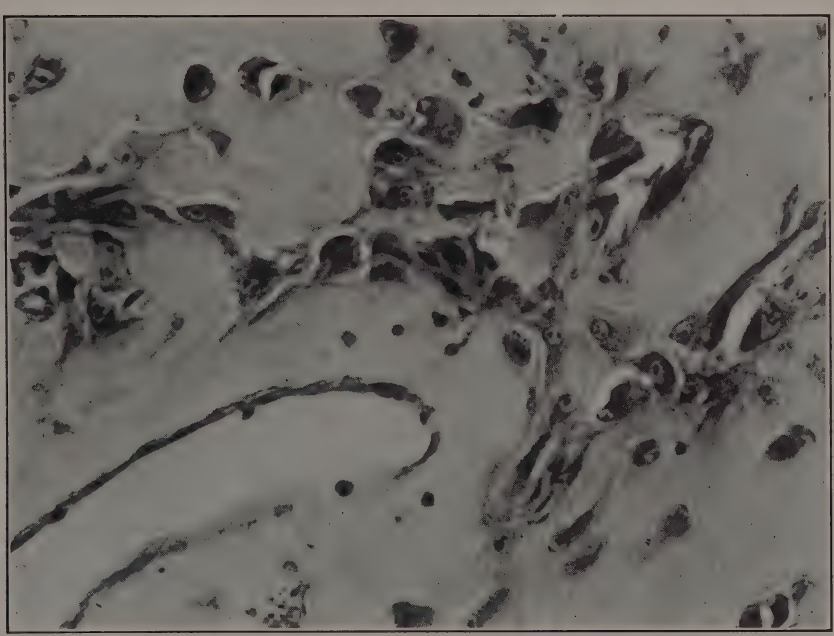


FIG. 10.

(Tytler: A Transplantable New Growth of the Fowl.)

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RESISTANCE TO A TUMOR-PRODUCING AGENT AS DISTINCT FROM RESISTANCE TO THE IMPLANTED TUMOR CELLS.

OBSERVATIONS WITH A SARCOMA OF THE FOWL.*

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PLATE 32.

No one of the problems opened to experiment by the transplantation of animal tumors has received more attention than that of the nature of resistance to these growths. The exact manner of the resistance, or resistances,—for there may well be several kinds,—is still undetermined; nevertheless one important fact has emerged, namely, that the fate of implanted tumor, as indicating this resistance, is influenced for the most part by the same conditions that influence the fate of implanted normal tissue. The conditions at the site of the graft's implantation, the age of the host, its health, species, even variety, and its relationship by blood to the previous host,—these and other factors influence in like manner engrafted tissue of both sorts.[1] Furthermore, resistance to transplanted tumor can be induced by a preceding injection of living tissue,[2] and whether this tissue be normal or neoplastic makes little difference.[3] By the same means too animals can be made resistant against implanted normal tissue which ordinarily would grow in them, for example embryonic tissue, so that it now fails to develop (Fichera, Rous). The numerous data thus far obtained go to show that the

* Received for publication, June 21, 1913.

1 Loeb, L., *Proc. Am. Phil. Soc.*, 1908, xlvii, 3. Fichera, G., *Arch. ed atti d. Soc. ital. di Chir.*, 1909, xxii, 1. Rous, P., *Jour. Exper. Med.*, 1910, xii, 344.

2 Ehrlich, P., *Arb. a. d. k. Inst. f. exper. Therap.*, 1906, No. 1, 77.

3 Bashford, E. F., Cramer, W., and Murray, J. A., *Brit. Med. Jour.*, 1906, i, 207. Schöne, G., *München. med. Wchnschr.*, 1906, liii, 2517.

resistance which in some individuals prevents the growth of implanted tumor is a resistance directed against the graft as strange tissue, and is unconnected with the neoplastic qualities which this tissue happens to possess.

These are the findings with transplanted neoplasms. Of the specific factors concerned in resistance to the growth of spontaneous tumors, as distinct from non-specific factors, such as infection, mechanical disturbance, etc., practically nothing is known. Manifestly these growths cannot be looked upon as consisting of a tissue strange to the body in the sense that transplanted tumor tissue is strange. The not infrequent occurrence of spontaneous tumors in animals highly resistant to implanted growths (Bashford) proves this.

The present paper is a report of an attempt to learn, through the study of an avian tumor, something of the nature of resistance to spontaneous growths. The tumor employed is a spindle-celled sarcoma of the chicken, which has already been reported upon several times from this laboratory.[4] The growth, when transmitted by transplantation, behaves exactly like the transplantable tumors of mammals, being influenced by the factors which influence them in common with transplanted normal tissue. But from it there can be separated by drying, or filtration, or glycerinization, an agent, presumably living, which, under special conditions, will cause a sarcomatous change in the tissue of a previously normal fowl. The growth thus generated is derived from the host's own tissues and in this regard resembles the spontaneous tumors of mammals. A study of the forces influencing such a growth's development, and the circumstances which sometimes bring about its retrogression may well have a bearing on similar problems in mammalian oncology.

Natural retrogression of the spontaneous tumors of mammals is relatively rare; but retrogression can be induced, more or less successfully, by the Roentgen rays, radium, and ultraviolet light. The point of attack of these forms of radiant energy is still unknown. In the case of the chicken sarcoma when influenced by radiant

[4] Rous, P., *Jour. Exper. Med.*, 1910, xii, 696; for references to other articles on the growth, see Rous, P., and Murphy, J. B., *Berl. klin. Wchnschr.*, 1913, l, 637.

energy there arises at once the question whether the sarcomatous cells as such are primarily affected, or whether the agent responsible for the growth is injured, with a resultant lessened malignancy of the cells so that they can be destroyed by the body. My first experiments have been directed to this matter. Certain differences in the time of origin of growths caused by the dried tumor tissue, that is to say by an action of the tumor-producing agent, and of those resulting from the fresh tissue containing transplantable tumor cells have suggested the method of work.

METHOD.

With parallel safety-razor blades the fresh sarcomatous tissue is cut into a number of slices of equal thickness, ordinarily 0.08 to 0.1 cm. These are placed in Ringer's solution, carefully trimmed free of all except sound tumor, and are then separated into a number of batches. If the tissue is to be submitted to the Roentgen ray the slices of each batch, spread flat, are together sealed in a large hollow-ground slide under a large, thin cover-glass. If the ultraviolet ray is to be used the slices are repeatedly washed with Ringer's solution to rid them as far as possible of free albuminous matter, and, covered with a thin layer of the fluid, are exposed to the light in an open dish; or without the fluid they are sealed between two flat pieces of quartz glass previous to exposure. The control is treated in the same way except that it is not irradiated. Radium has not been employed thus far.

When the exposures have been completed the tissue is cut fine with sterile knives. In some of the experiments a little sterile infusorial earth was added to it. By means of trocars small portions (about 0.01 c.c. each) are now inoculated intradermally in the feather-free pectoral strip of a number of chickens. The remainder of the tissue is spread very thin in a dish, and placed *in vacuo* over sulphuric acid for twenty-four hours, which is sufficient time to render it completely dry. It is then restored to approximately its former bulk by the addition of an excess of Ringer's solution, or a very little distilled water, and inoculations are made with it as on the preceding day, using the same chickens and the feather-free strip of the other breast. Usually five batches of irradiated tissue and a control batch have been used, fresh and dry, making in all twelve inoculations to each fowl. The tissue bits are implanted several centimeters apart, in a line, and their order is varied from fowl to fowl. The tumors resulting are extremely discrete and are plainly visible at all stages of their development.

An Heraeus mercury-quartz lamp of 220 volts supplied the ultraviolet rays. The specimens were exposed at 25 cm. distance. The temperature of the preparations was at no time above 28° C.

For the Roentgen rays one or another of three soft tubes with a spark gap of 1.5 to 4.5 cm. has been employed. The specimens were placed within 5 to 10 cm. of the tube.

Five sets of observations with the ultraviolet rays and four with the Roentgen rays have been made by this method. For each experiment three to seven fowls were used, making in all forty-one inoculated, of which forty developed tumors. Retrogression soon occurred in some.

The growths from the inoculation of fresh material in intradermal sites arise in general almost immediately, resulting, as previous work has shown,[5] from an extremely rapid proliferation of the implanted cells. At the end of a week the little tumors may be one centimeter in diameter. They appear as raised, translucent bosses or buttons over which the epidermis is tense and smooth (figures 1 and 2). At the end of two or three weeks they very commonly ulcerate, or coalesce, so that observations beyond this period are not of great value. The growths from the dried material are exactly similar but appear much later, not until at least seven days have elapsed, and often twice or thrice this time. That desiccation completely kills the tumor cells seems certain from the findings of previous workers with the normal and neoplastic cells of the higher animals. Moreover, experiments by Dr. Murphy show that the embryonic tissue of the fowl does not survive drying; and drying renders completely innocuous another transplantable chicken tumor (Chicken Tumor XVIII), propagated in this laboratory.

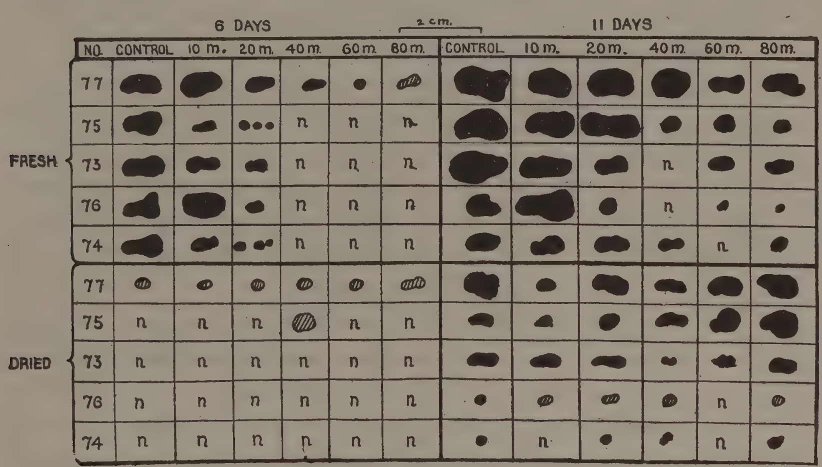
EFFECTS OF THE IRRADIATION.

The experiments have given almost diagrammatic results (text-figures 1 to 6). The Roentgen rays do not appreciably affect either the sarcoma cells or the growth's causative agent when the tissue has been exposed for eighty minutes, the longest time employed. This failure to be influenced is not surprising when it is considered how refractory many mammalian sarcomata are to the rays. The protocols of the individual experiments will not be given.

Ultraviolet light rapidly destroys the activity of the sarcoma cells and this without notably injuring the agent associated with them. In text-figure 1 its selective action is shown. At the end of six days good sized growths are found as the result of inoculation of the fresh control material, while the irradiated fresh tissue mani-

[5] Rous, P., and Murphy, J. B., *Jour. Exper. Med.*, 1912, xv, 270.

fests less tumor-producing activity, until with sixty and eighty minutes' exposure this is completely suppressed. The dried material has as yet given rise to no growths. At the end of eleven days the effects of irradiation and of drying are less marked. In four of the five fowls the dried material has given rise to growths; and in the size of these growths there is no evidence of influence of the irradiation. Differences among the tumors from the fresh material still

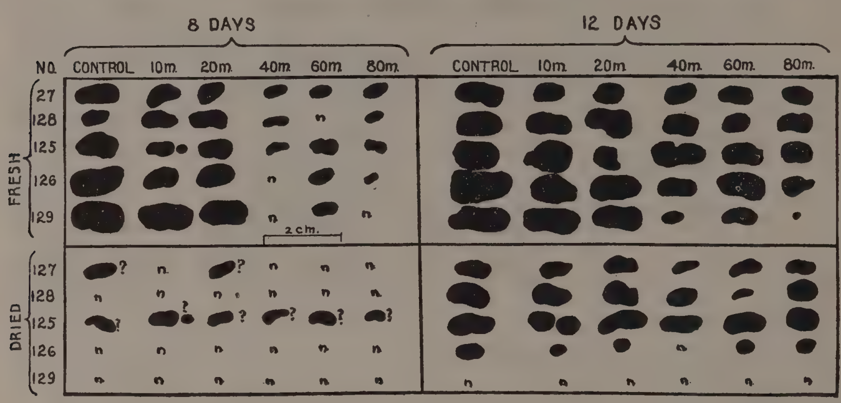


TEXT-FIG. 1. To show the effect of ultraviolet light on the activity of fresh and dried tumor tissue. In the first vertical column are the numbers designating the fowls. Then follow silhouettes to scale of the tumors. The measurements were taken six and eleven days after the inoculations. 10 m., 20 m., 40 m., etc. = 10, 20, and 40 minutes' exposure; n = no tumor. Cross-hatching = induration.

It will be seen that the irradiation has a marked injurious effect on the element in the fresh tissue (the living tumor cells) which gives rise to tumors immediately after implantation. The tumor-producing agent which resists drying is unaffected by the irradiation.

indicate this influence, but less strikingly than before, since now at length the material irradiated for sixty and eighty minutes has given rise to growths. These growths have arisen after about the same time and are of about the same size as those derived from the dried material. In text-figure 2 similar findings are presented but the differences are not so clear cut, since the longest period of irradiation has failed to rid the fresh tissue of the activity specifically associated with its fresh state.

The interpretation of these results is plain. The text-figures show, first, that in the sarcomatous tissue there are two elements capable of producing the growth, one of which will withstand drying while the other will not. The labile element, which we know to be the living and transplantable tumor cells, is so sensitive to the ultraviolet rays that sixty to eighty minutes' exposure will completely destroy its activity; whereas the stable element, the tumor-producing agent, is at most only slightly affected by this irradiation.



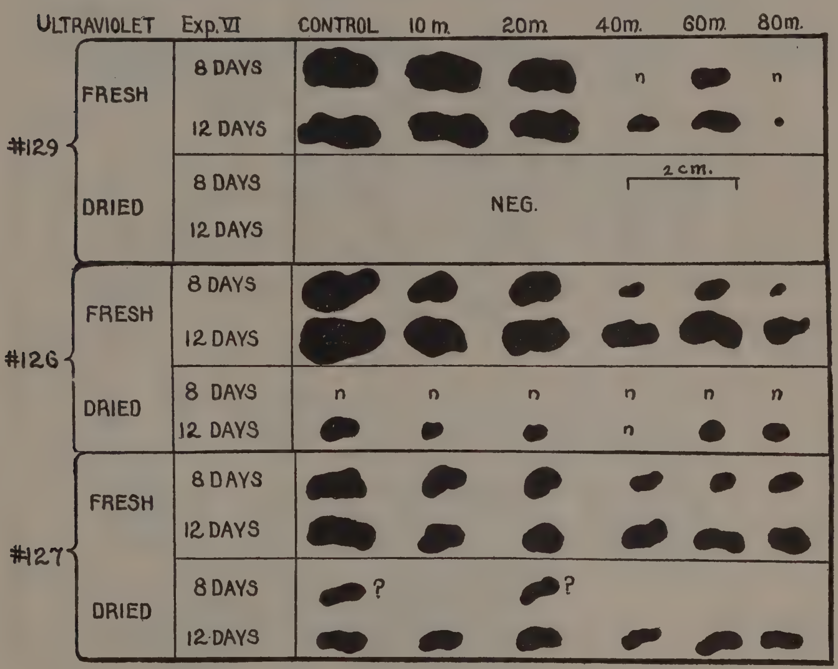
TEXT-FIG. 2. This chart is arranged in the same way and shows findings similar to those of text-figure 1.

TYPES OF RESISTANCE TO THE SARCOMA.

Taken from another point of view the irradiation experiments have demonstrated the presence in the fowl of two distinct resistances to the chicken sarcoma, directed, the one against the tumor cells, the other against the tumor-producing agent. These may exist separately or in one host.

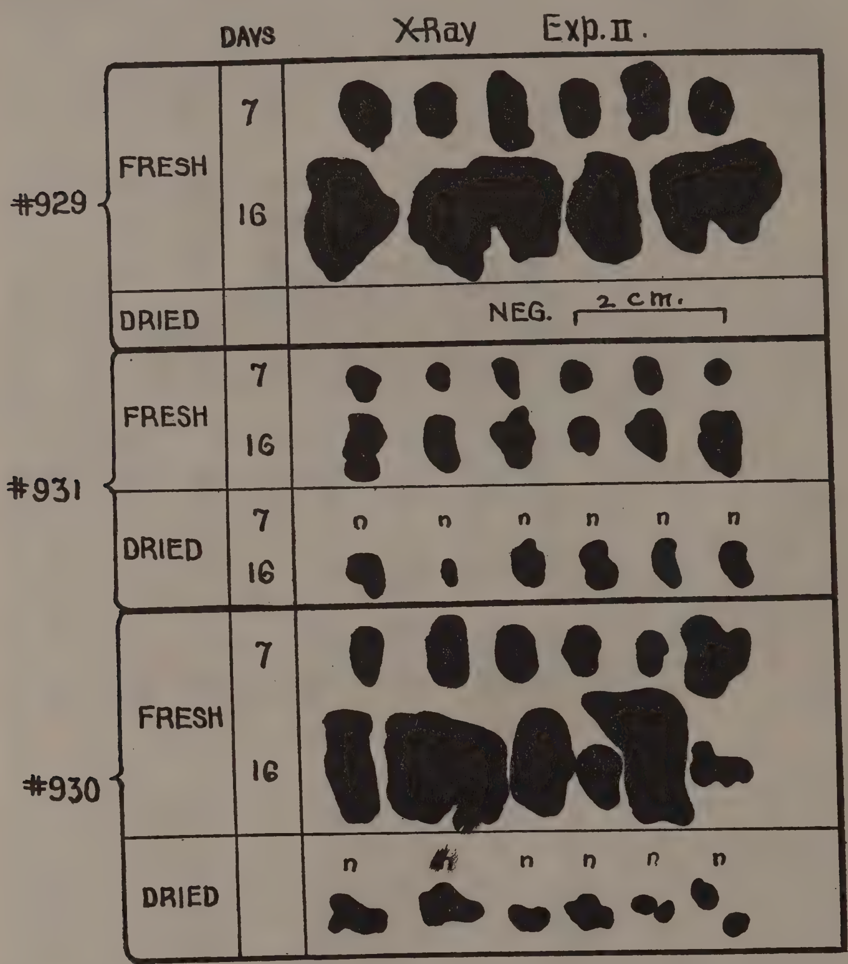
In text-figure 3 are given the findings in three of the fowls of text-figure 2 but now so arranged as to bring into contrast the fate of the fresh and dried material in the individual host. The fresh material produced tumors in all three fowls but with a very different degree of success. In one instance, No. 129, in which it gave rise to large tumors the dried material failed to engender growths, and in another, No. 126, this latter acted only slowly. In the remaining

instance growths developed rapidly from the dried material and the fresh material proved only slightly more active, the results with it being poor as compared with those in the first two hosts. In text-figure 4 precisely the same facts are shown of some fowls of X-ray experiment II, and here the complicating effect of a variation in tumor size due to injury from the irradiation is not



TEXT-FIG. 3. Some of the results shown in text-figure 2, but now so arranged as to demonstrate individual differences in the resistance of the fowls and the presence of two sorts of resistance. In No. 129 the fresh tissue rapidly gave rise to tumors, whereas the same material, when dried, failed to engender them. That this is not due to lack of activity on the part of the dried stuff is shown by the findings in the other two fowls. In fowl 126 the fresh tissue rapidly engendered tumors and the dried material in due time caused them. In No. 127 the dried material was active in the production of growths, whereas the fresh material was much less successful than in the fowls previously mentioned. It is evident that some hosts have a relative resistance against a tumor-producing element in the fresh tissue, while in some a resistance is directed against the tumor-producing element which survives drying. ? = possibly a tumor, possibly only induration.

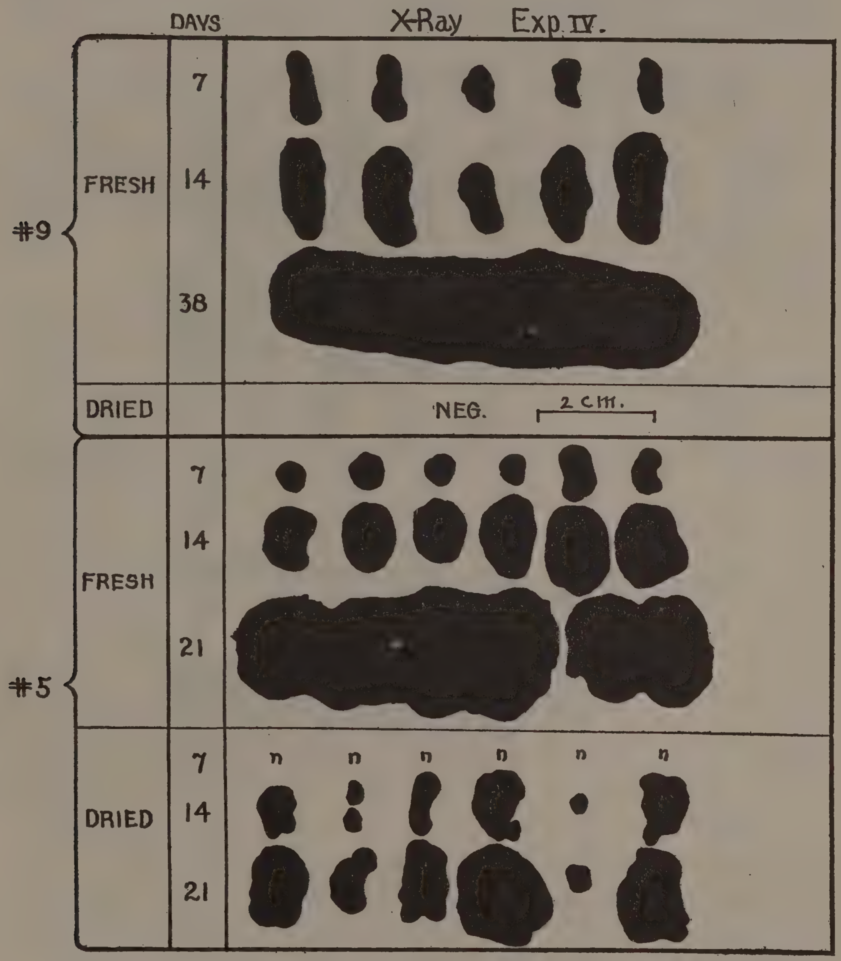
present. In text-figures 5 and 6 some phases of the matter are again illustrated. The fowls in which the dried material is very active



TEXT-FIG. 4. This illustrates the same facts as text-figure 3. The periods of exposure to the X-rays are not given since the material was practically unaffected by them.

are not necessarily those in which the fresh material does badly, but often ones in which it does very well. Other charts showing the same facts might be given. The chickens of all the experiments

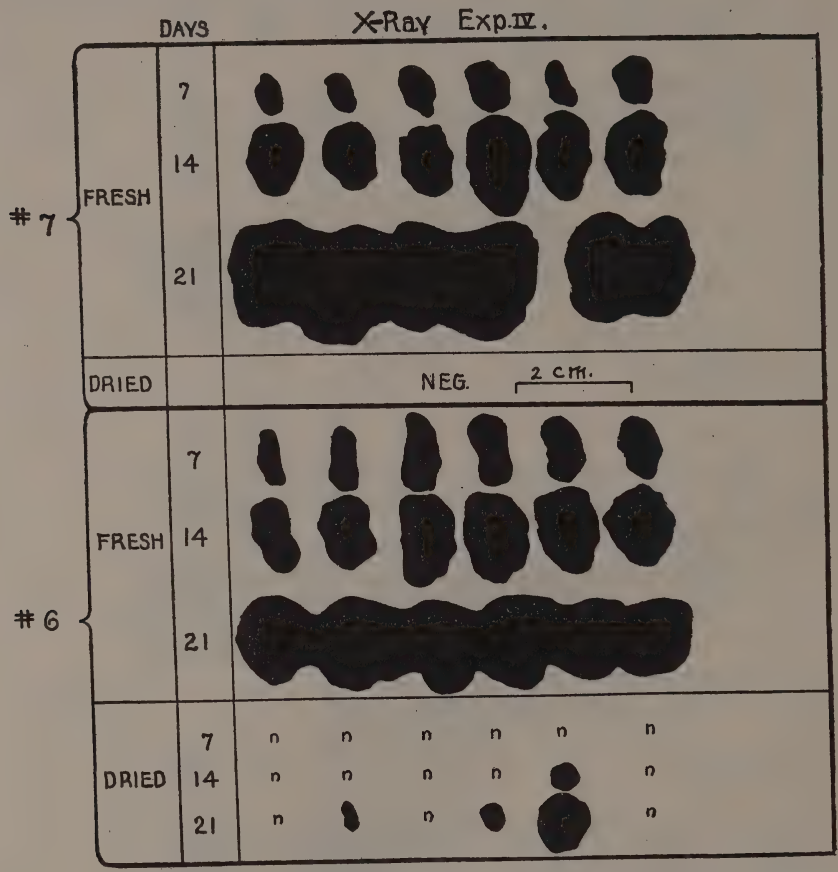
can be separated into four classes: those in which the fresh material gave rise to tumors and the dried gave none; those in which both



TEXT-FIG. 5. This illustrates further some of the facts of text-figures 3 and 4 and shows that good growth from dried material is not necessarily associated with poor results from the fresh tissue.

gave rise to tumors with nearly equal facility; others again in which neither gave rise to growths; and finally a majority in which both

inoculations resulted in growths, those from the fresh material appearing earlier and maintaining a marked general advantage of size. In other words the chickens showed themselves resistant or susceptible in widely various degrees to one, or another, or both the



TEXT-FIG. 6. This chart shows again the differences in individual fowls.

labile and stable elements going to produce the tumor. And we know that these labile and stable elements are, respectively, the tumor cell and the tumor-causing agent.

A number of questions at once present themselves in relation to this conclusion. Resistance to the transplanted tumor cells is never

so clear cut as that to the agent. It is at best only relative. If fowls are really resistant to the implanted cells how does it happen that even in hosts most resistant to them growths from the fresh material appear somewhat earlier and at first are larger than those from the dried material? One reason is because in resistant fowls a profuse, local, round-celled reaction occurs about the fresh tissue implanted in intradermal sites, forming transiently a considerable nodule.[6] Perhaps also the tumor-producing agent is somewhat attenuated by drying. Theoretically the findings, in fact all of the differences between fresh and dried material, might be explained on the assumption that the chicken sarcoma is never really transplanted but comes only from infection by means of an agent largely impaired by drying. This is contrary to the facts and entails awkward secondary assumptions, for example, that fowls react differently to the stable and labile portions of the agent, some being susceptible only to the one, some to the other. But we know that the differences between fresh and dried material are primarily those of the tumor cells involved. Furthermore, investigations have shown that some fowls are naturally resistant to the action of the tumor-producing agent[7] and some to growth of the implanted tumor cells.[8] The present work goes only a step further in demonstrating that the resistances thus manifested are independent of one another.

It is interesting to consider in the light of these results the rôle of the causative agent in the growth of the sarcoma in the individual fowl. Histologically there is no suggestion that the agent takes part in this process. And yet in one class of susceptible fowls, as the present results show, it doubtless aids in the growth's extension. In another class the tumor's development following the implantation of tumor cells is probably from the first solely the result of the proliferation of these cells.

In the present state of our knowledge it is impossible to say whether findings with chicken tumors have a direct application to

[6] Rous, P., and Murphy, J. B., *Jour. Exper. Med.*, 1912, xv, 270.

[7] Rous, P., Murphy, J. B., and Tytler, W. H., *Jour. Am. Med. Assn.*, 1912, lviii, 1751.

[8] Rous, P., *Jour. Exper. Med.*, 1910, xii, 696; Rous, P., and Murphy, J. B., *Berl. klin. Wchnschr., loc. cit.*

the problems of mammalian tumors. Were this so then the present observations would go far to explain how it happens that "spontaneous" growths can arise, as they sometimes do, in rats or mice highly resistant to transplanted neoplasms. For in these individuals resistance to implanted cells need by no means carry with it resistance to a tumor-producing agent.

SUMMARY.

Ultraviolet light rapidly kills the cells of a transplantable sarcoma of the fowl without notably injuring the etiological agent associated therewith. The Roentgen ray has little effect on either cells or agent.

Fowls manifest two sorts of resistance to the avian tumor, one directed against the implanted tumor cells as such, the other against the action of the etiological agent to cause a neoplastic change. In the individual fowl the two resistances appear to be independent of one another, though they may exist together or may both be absent. A recognition of them will perhaps explain some features in the biology of other tumors.

The work has been done with the assistance of Dr. Linda Lange.

EXPLANATION OF PLATE 32.

FIG. 1. A photograph taken eight days after the implantation of material exposed to the Roentgen rays, showing the discrete character of the intradermal tumors. Tumors have arisen, thus far, only from the fresh material. The dried tissue was put in the left pectoral strip. The large mass to the left of the upper end of the sternum is the crop.

FIG. 2. Intradermal tumors in the pectoral strips. The preparation has been turned so that the sternal keel, if present, would lie horizontally along its middle.

The upper line of growths (right pectoral strip) has resulted from the inoculation of fresh tumor tissue exposed in a glass tube to the ultraviolet light for various periods. In the lower line are growths from some of the same material exposed in the same way but in a quartz tube. At the extreme left of each line (left of the photograph) is a tumor from the control material. The periods of irradiation become longer as one proceeds to the right. The photograph was taken on the seventeenth day after the inoculations.

It will be seen that the ultraviolet rays have failed to act through glass, but through quartz their action has been such that at the end of seventeen days tumors are just beginning to appear from the material irradiated longest (fifteen and thirty minutes).

FIG. 1.

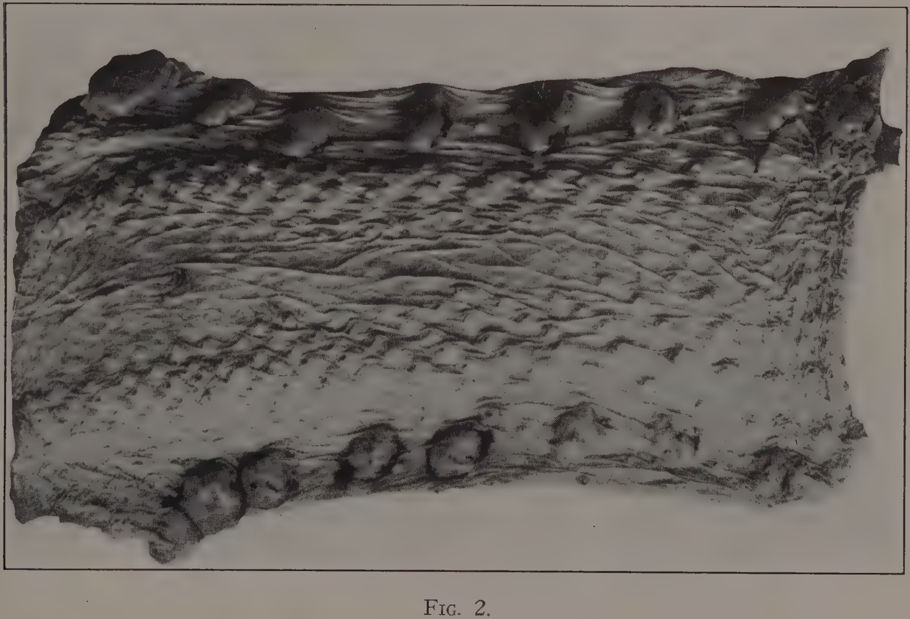


FIG. 2.

(Rous: Resistance to Tumor-Producing Agent.)

[Reprinted from the Journal of Experimental Medicine, Vol. XVIII, No. 6, 1913.]

THE CHARACTERS OF A THIRD TRANSPLANTABLE CHICKEN TUMOR DUE TO A FILTERABLE CAUSE. A SARCOMA OF INTRACANALICULAR PATTERN.*

By PEYTON ROUS, M.D., and LINDA B. LANGE, M.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research, New York.*)

Plates 49 to 55.

That tumors of the fowl are far from rare is now recognized; and there exists a considerable literature concerning them.[1] Efforts to transplant the growths have met in general with poor success. Thus far only three transplantable chicken tumors have been described. Three others are at present under propagation in our laboratory, one of which forms the subject of the present paper. This tumor owes its transplantation to the use of many fowls as hosts. Probably the failure to transfer chicken tumors has often been due to neglect of the point. If only two or three animals were used in each attempt to transfer spontaneous mouse tumors the results would certainly be unsatisfactory.[2]

The three fowl tumors previously described are a spindle-celled sarcoma,[3] a myxosarcoma,[4] and an osteochondrosarcoma.[5] The first and last of these growths have been reported upon in detail.

* Received for publication, July 3, 1913.

[1] Ehrenreich and Michaelis, Tyzzer, Rous, Murphy, Wernicke, Tytler.

[2] Some tumors of the fowl, like some tumors of the mouse, resist all attempts at transplantation. The epitheliomata of the scaly portion of the leg are among these. During the past two years five such spontaneous epitheliomata have been brought to the laboratory; and more than one hundred young adult chickens have been inoculated from them, often in several ways, but without success.

[3] Rous, P., *Jour. Exper. Med.*, 1910, xii, 696.

[4] Fujinimi, A., and Inamato, K., *Verhandl. d. Jap. path. Gesellsch.*, 1911, 114.

[5] Rous, P., Murphy, J. B., and Tytler, W. H., *Jour. Am. Med. Assn.*, 1912, lviii, 1682; Tytler, W. H., *Jour. Exper. Med.*, 1913, xvii, 466.

They have interest not only as characteristic neoplasms in the bird, but because the cause of each has been found in an agent separable from the tumor tissue by filtration through a Berkefeld filter.[6] The growth now to be described is quite as characteristically a neoplasm and also has a filterable cause. The experiments upon its etiology will be published in a separate paper. It is a connective tissue tumor, a sarcoma split and fissured by many compressed blood sinuses into which it tends to extend and grow in an intracanalicular pattern. Its like has not been found among the forty-three spontaneous chicken tumors recently studied by us.

THE ORIGINAL GROWTH.

The fowl with the spontaneous tumor was brought to us because of swellings in the left leg that rendered it lame. The swellings proved to be two smooth and very firm masses, lying in the muscle above and below the knee joint. They seemed attached to the bone and limited the movement at the joint in both directions. The fowl, a mongrel, brown Leghorn hen, was sparely nourished; it died under chloroform during an exploratory operation. Inoculation of portions of the tumors were made within three hours into ten Leghorn chickens.

Gross Findings.—In the gizzard was a mass which may with good reason be regarded as the primary growth. It occupied nearly the whole right anterior portion of the organ, and projected irregularly under a covering of mesentery (figure 1) in which lay several small nodules up to 0.6 cm. in diameter, some sessile on the gizzard, some free. On section the mass proved roughly spherical, measuring 4.3 by 4.3 cm. (figure 2). It was everywhere surrounded by muscle and though it encroached on the gizzard cavity the mucous membrane was not eroded. It had no capsule but was sharply demarcated by its color, a pale, pinkish white, from the wine-colored muscle. It bulged on the cut surface and was made up of many irregular, tightly compressed subdivisions separated by minute, irregular fissures. Toward one side was a localized soft, yellow necrosis, but the growth was in general translucent and, though poorly vascularized, appeared sound. In the fold of mesentery extending from the gizzard to the left lobe of the liver was a flattened grayish pink nodule measuring 0.2 by 0.05 cm.

The liver was enlarged, congested, mottled with ill defined, pale areas. On its anterior surface were two small, stellate depressions marking irregular, gray areas in the parenchyma that suggested scars. These areas were later found to consist of tumor tissue. The other viscera appeared normal.

In the substance of the extensor muscles of the left thigh was an oblong, smooth mass 4.5 by 3 by 3 cm. with its long diameter in the direction of the muscle. A similar mass 4 by 3 by 3 cm. occupied the muscles just below the knee (figure 3). The upper mass was firmly attached to the periosteum for

[6] Rous, P., *Jour. Am. Med. Assn.*, 1911, lvi, 198; *Jour. Exper. Med., loc. cit.;* Rous, P., Murphy, J. B., and Tytler, W. H., *Jour. Am. Med. Assn.*, 1912, lix, 1793.

about 2 cm. above the patella, and to the joint capsule also. A prolongation extended from it around to the back of the joint. The lower mass was attached to the joint capsule but the bulk of it lay in the muscle. The growths were extremely firm, much firmer than the one in the gizzard, pinkish white, nearly bloodless, and consisted of finely striated tissue, in part solid and in part, like the tumor of the gizzard, divided by fissures into irregular lobuli. Some of the lobuli had a central, semigelatinous depression, and in a few the center was yellow, firm, and opaque,—evidently necrotic. The growths were unencapsulated and poorly defined from the muscle sheaths, periosteum, and tendons which they involved.

The knee joint was full of papillary proliferations (figure 3), translucent and gristly, attached to the capsule, or joint surface. The largest one had its base on the posterior surface of the patella. Microscopically all were found to be extensions from the growths on the outer surface of the capsule. Within the lower end of the femur, lying in the red marrow, was a translucent tumor mass about 0.4 cm. in diameter. The bone from without had seemed normal.

In the muscle of the chest wall just anterior to the junction of the sternal and vertebral portions of the three upper right ribs was a tumor measuring 2.5 by 1.4 by 1 cm., which projected into the thoracic cavity (figure 1). In the anterior muscles of the neck, just above the left clavicle, was a similar mass, 3.9 by 1.7 by 1.5 cm. in size; and yet a third, measuring 3 by 2 by 1 cm., lay in the muscle of the inner surface of the pelvis near the right anterior portion of its rim. All resembled the growths in the leg.

Microscopic Findings.—The tumor of the gizzard consists of a spindle-celled tissue irregularly split and separated into islands by many fairly wide channels now quite empty except for a few blood cells (figure 4). The channels are walled by only a single layer of endothelium and some of the smaller ones lack even this and are probably artefacts due to shrinkage in the fixing fluid. The prominence of the larger channels is doubtless dependent to some extent on the same cause, since they are nearly empty and the tumor in the gross was practically bloodless.

Mitoses are fairly frequent. The tumor's enlargement has obviously taken place by intrinsic proliferation coupled with the direct invasion and replacement of normal structures. In the muscle just beyond the growth's border, and not yet attacked, are numerous dilated blood spaces and capillaries. Within the tumor the sinuses thus formed have become more extensive but are filled for the most part with papilliferous extensions of tumor tissue covered everywhere by endothelium. Many of the sinuses are compressed to mere slits.

The metastases in the muscles (figure 5) closely resemble in the main the growth in the gizzard. In some parts, however, sinuses are infrequent and the tissue is practically solid.

Briefly, the curious tumor,—called in this laboratory Chicken Tumor XVIII,—consists of a spindle-celled, sarcomatous tissue fissured and subdivided by many flattened sinuses, and often intracanalicular in its growth (figures 4 and 5). The primary mass, as

the later findings have shown, lay in the wall of the gizzard (figures 1 and 2). Secondary growths were present at several points in the skeletal muscles, notably in the left leg where were two large masses, one of which had extended into the knee joint and the marrow of the femur (figure 3).

TRANSPLANTATIONS.

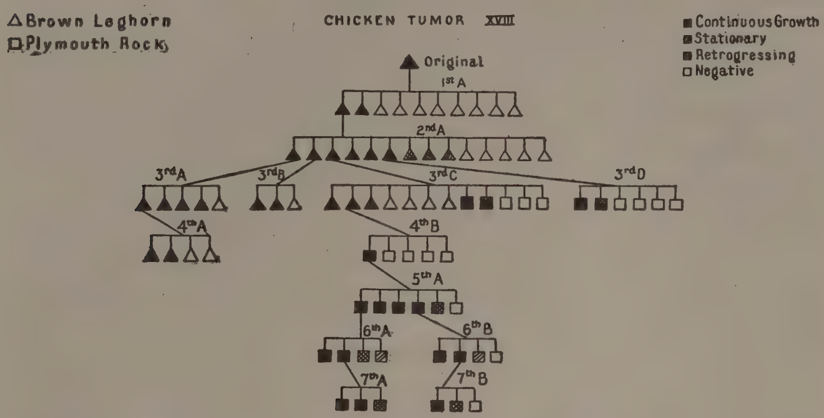
Transplantations of the tumor were made three hours after the host's death, by the insertion through a trocar of small bits of the fresh tissue deep in the pectoral muscle of normal fowls. The tissue used was taken from one of the growths in the left leg. The fowls were young brown Leghorns, chosen for their similarity to the original fowl, since previous observations had shown[7] that the success of transplantation sometimes depends on the use of chickens of the same strain as the original. The fowls received two implantations, one in each breast.

Growths developed in only two of the chickens and in one of these eventually retrogressed. The nodules became palpable within a month, but their enlargement was extremely slow. That they were the expression of a general susceptibility is shown by their development at both sites of inoculation. Material for the second tumor generation was obtained by removing, forty-six days after inoculation, the tumor largest at this time. A recurrence promptly took place at the site of operation but its growth was slow. Both fowls were alive and active eight months after inoculation, one with growths still retrogressing from a maximum diameter of two centimeters, the other with masses that had gradually increased to five centimeters across. Both were now inoculated in the leg muscles with another and more malignant chicken tumor (Chicken Tumor I, a spindle-celled sarcoma) and of this they eventually died, though not till one had rounded out nearly a year as tumor host.

Chicken Tumor XVIII is now in its eighth tumor generation. It has been pushed thus far in fifteen months by transplanting from the sarcomatous nodules shortly after their appearance. With repeated transfer the percentage of successful inoculations and the rate of growth have increased markedly (text-figure 1). In the earlier generations care was taken to select hosts of brown Leghorn

[7] Rous, P., *Jour. Exper. Med., loc. cit.*

strain. Though the percentage of takes increased, the growth of the tumor was still slow when, for the third generation, series C, five brown Leghorn and six Plymouth Rock fowls were inoculated. In two hosts of each sort tumors developed, and in one Plymouth Rock they grew with unexampled rapidity. Retransplantation to other



TEXT-FIG. I. Results of the transplantation of Chicken Tumor XVIII into seven consecutive series of fowls. The percentage of successful inoculations has been much increased, and seems little influenced by the variety of the host.

Plymouth Rock fowls resulted in a still further increase in rapidity of growth and some changes in structure of the tumor and distribution of metastases. In the absence of direct comparative tests it cannot be said that the findings in Plymouth Rocks represent a special susceptibility of these fowls, because similar results might have followed a fortunate transplantation in brown Leghorns. But the facts show that Chicken Tumor XVIII, like Chicken Tumor VII (an osteochondrosarcoma), and unlike Chicken Tumor I (a spindle-celled sarcoma) in its earlier generations, can be readily transplanted to fowls of another variety than that in which it occurred spontaneously.

BEHAVIOR OF THE TRANSPLANTED TUMORS.

The usual history of one of the tumors of the early generations in Leghorn fowls is as follows:

Two or three weeks after the implantation a small, firm nodule

appears where the needle has been thrust deep in the breast. This slowly enlarges with its long axis in the direction taken by the muscle fibers. On section it consists of an extremely firm, bulging, finely striated, grayish pink tissue, nearly bloodless, and in part finely subdivided by minute fissures. Small areas of necrosis or hemorrhage may be present. There is no capsule. Enlargement of the nodule takes place so slowly that only after from four to seven months does it attain a diameter of five centimeters. It is still extremely firm and somewhat elastic, on palpation suggesting cartilage, and is sound practically throughout. The host though still active emaciates as the mass becomes large and the wasting of the pectorals throws the tumor into relief. Its surface is smooth and it is irregularly football-shaped or shaped like a flattened sphere. The cut surface may be finely fissured, but there is little indication of the elaborate, intracanalicular arrangement visible macroscopically. Rarely the skin over the tumor becomes attached, or the growth fixes itself to the sternum. In resistant hosts the mass is encapsulated and may show a gross lobulation.

When the tumor has become large, secondary growths appear in the limbs of some hosts. The fowl limps and its wings seem stiff. On examination hard, elastic masses are found lying as a rule in the muscle above or below the knee joint anterior to the bone, and near the first joint of the wing on its under surface. They sometimes occur in the muscles of the neck, and in the intercostals, and about the hip. There are often several but never many of the secondary growths in one fowl, and each seems to arise by enlargement from a solitary focus. They start near a joint as a rule, sometimes from the outer surface of its capsule, and extend along and within the muscles and tendons as discrete, smooth, firm masses of much larger bulk than the normal tissues which they replace. They hinder movement greatly, and by binding the muscles to the periosteum may practically prevent it. The points at which they appear in the wing and leg are those much exposed to trauma.

The fowl in which secondary growths have developed presents a truly remarkable picture (figure 6). The wings, held close, in nearly the normal position, are not paralyzed but are stiffened,

sometimes to immovability, by firm masses lying in the muscle substance. The legs are greatly swollen about the knee and are nearly immovable at this joint. To either side of the sternum are the large, projecting, primary growths. With all this the fowl may be vigorous; and the growths give it a factitious plumpness. But sooner or later it goes into coma and a day or two later dies.

The viscera are usually free from metastases. A few small nodules may be present in the lungs. In one instance they were found in the heart. In a fowl of the second tumor generation, dying between five and six months after inoculation, there were, in addition to metastases in the wings and legs, several localizations in the skin of the body, appearing as plateau-like placques, or thickenings, raised three or four millimeters above the surface. These placques were roughly oval, circumscribed, and consisted of tumor tissue growing in the loose, superficial layer of the corium and limited by its dense, sheet-like, deep layer. The epidermis over them was not ulcerated or discolored, but the pitting of the feather follicles was much exaggerated. The viscera in this case were free of tumor.

At every autopsy the gizzard has been carefully examined. Secondary growths have not been found in it. That the tumor in the gizzard of the original fowl was primary and those in the muscles metastases seems certain.

In the Plymouth Rock fowls of the fourth to eighth tumor generations the tumor sometimes grew so rapidly as to measure five centimeters across one month after the inoculation, shortly occupying the entire breast. It has retained its firm, dense character, but no longer gives metastases to the limbs. Metastases have been found several times in the lungs, less often in the heart and liver, and once in the omentum; but in general secondary growths are rare. Those in the lungs may coalesce to render the organ semisolid.

HISTOLOGY.

In the earlier generations (brown Leghorn fowls) the tumor retained the structure of the spontaneous growth. The fissuring was often very marked and intracanalicular extension not infrequent (figure 7). Both features were found in primary and metastatic

growths, situated in the voluntary muscles; but in growths of the lungs, heart, and skin they were absent, and the tissue was that of a pure, spindle-celled sarcoma (figure 8). The structure of the organ involved, and the peculiar fashion of the growth's invasion, as interacting factors, are doubtless responsible for this difference. In its method of invasion and replacement, as exemplified in voluntary muscle, Chicken Tumor XVIII differs from other chicken tumors thus far studied. Leaving out of consideration the method of pressure atrophy common to all, Chicken Tumor I, a spindle-celled sarcoma, destroys the muscle, either by a direct apposition of tumor cells to its fibers, associated with what may be called erosion, or by a direct extension into the interior of the muscle fibers and proliferation there. Figures illustrating this have been published.[8] Chicken Tumor VII, the osteochondrosarcoma, replaces the muscle in both ways, doing so usually by the first. But Chicken Tumor XVIII, by means of strands of proliferating cells, splits up each muscle fiber longitudinally into a number of fragments which are then absorbed (figure 9). In cross-sections of the muscle at a certain period in the process of replacement a hundred isolated muscle bits, each apparently an atrophying unit, lie in the space normally occupied by twenty or thirty muscle fibers. They are derived indeed only from this number. During the replacement the preëxisting blood spaces enlarge; intracanalicular growth into them takes place to greater or less extent; and there results a tissue irregularly subdivided by blood sinuses, and often to a large extent lying within them. The sinuses are always greatly compressed and nearly empty of cells. Many of the smaller clefts are not lined by endothelium, and are presumably artefacts due to shrinkage.

The extension of the tumor by invasion and replacement is more obvious than by expansion. Bone may be rapidly eroded. The firmness of the tissue is traceable to the large quantity of collagen distributed in it, often as thick, undulating ribbons or bundles. Next to these bundles the cells may be grouped regularly, in strata, but generally they lie in irregular strands or whorls, and the collagen fibers are irregularly distributed. In the more rapidly growing areas mitotic figures are frequent. There is a noteworthy

[8] Rous, P., and Murphy, J. B., *Jour. Exper. Med.*, 1913, xvii, 219.

absence of giant cells. The cells have considerable uniformity but vary somewhat in size. The nucleus is large and vesicular, the cytoplasm scanty, and the cell outline that of a short spindle. More or less attenuated cells are frequent, and occasionally very slender ones are seen, grouped in strands, with rod-shaped nuclei, suggesting to some extent smooth muscle. Focal hydropic changes occur (figure 10) and rarely a mucinous degeneration.

In the later generations of the tumor, those of its rapid growth in Plymouth Rock fowls, it is usually a pure, spindle-celled sarcoma, fissured by a few flattened sinuses. It has become more cellular, though collagen fibrils still render it firm. Degenerative forms are still absent.

In very susceptible fowls no cellular reaction is found at the tumor's border except that associated with its support and vascularization. Always there is a total absence of histological features suggesting infection. In fowls relatively resistant, as shown by the tumor's slow growth, bands of scar tissue divide it irregularly, and small round cells, with some macrophages, are massed at its border and scattered within it. The neoplastic tissue disappears, not by necrosis *en masse,* but by a gradual absorption which is compatible with survival of the cells for a long time. The histological phenomena are quite similar to those associated with the retrogression of mammalian tumors[9] and have been described at length in connection with another neoplasm of the fowl.[10]

RESISTANT HOSTS.

The tumor has not grown in pigeons, rats, or mice. Ten individuals of each sort were inoculated with material that was active, as shown by the results in chickens. The tumor fails to grow in some chickens of the susceptible varieties, and in others, after a greater or less development, it retrogresses. The question whether retrogression results in a heightened resistance, has not been settled. Fowls in which the tumor is growing can be successfully reinoculated.

[9] Da Fano, C., *Ztschr. f. Immunitätsforsch., Orig.,* 1910, v, 1.
[10] Rous, P., and Murphy, J. B., *Jour. Exper. Med.,* 1912, xv, 270.

TRANSPLANTABILITY.

In studying the transplantability of the tumor the fate of bits of it implanted intradermally has been followed. In some fowls a suspension of the tumor tissue has been injected intravenously and the lungs examined later in serial section, but these inoculations, so successful with Chicken Tumor I, have not resulted in tumors. Similar negative results from intravenous inoculation are recorded of several neoplasms of the rat and mouse.[11] The intradermal method has proven very satisfactory. Eight to ten tumor bits are implanted in each of a number of fowls, and are removed on successive days with the tissue about them, and submitted to serial section. In susceptible hosts the implanted cells survive, are vascularized, proliferate, and in a short time give rise to little tumors (figures 11, 12, and 13). The tissue, in other words, is transplantable. In resistant chickens the implanted material survives for a few days and may be vascularized, but perishes shortly in a mass of small, round cells. The findings will not be taken up in detail since they resemble those described for Chicken Tumor I.[12]

DISCUSSION.

The growth described is in its structure, its method of enlargement, of retrogression and in its transplantability a tumor. Corroborative evidence of its neoplastic character is found in its effect on the host, in its limitation in transmissibility to one species, and in the complete natural resistance manifested by some individuals of the susceptible species. Several unsuccessful attempts have been made to cultivate from its tissue an etiological organism. The cultures either have remained sterile, or have shown diverse contaminations, such as sooner or later enter a growth transplanted without rigorous asepsis.

Every tumor has its peculiarities and in one particular Chicken Tumor XVIII is nearly unique. It tends to metastasize to the skeletal muscles, and this without localization in the viscera, and in

[11] Graf, R., *Centralbl. f. allg. Path. u. path. Anat.*, 1910, xxi, 723; Daels, F., *Arch. de méd. expér. et d'anat. path.*, 1910, xxii, 645; Levin, I., and Sittenfield, M. J., *Proc. Soc. Exper. Biol. and Med.*, 1910–11, viii, 114.

[12] Rous, P., and Murphy, J. B., *Jour. Exper. Med.*, 1912, xv, 270.

the absence of a patent foramen ovale or other short cut to the greater circulation. The facts might be taken to suggest that the growth's distribution occurs, not by cell emboli, but by means of some minute, infective agent, to which the pulmonary capillaries offer no barrier. As a matter of fact the paradoxical distribution of the metastases speaks neither for nor against an origin by cell emboli. It is true that pulmonary metastases are often the first step to the distribution of a growth by the greater circulation; and their presence furnishes good evidence for the dependence of this process upon cells: yet carcinoma and sarcoma both succeed rather frequently in passing the barrier of the pulmonary capillaries without the aid of lung tumors, so far as can be determined.[13] Everyone with an experience at the autopsy table has met such cases. The slight recognition accorded them in text-books perhaps results from the endeavor to present in a sharp cut way the mechanics of cell distribution and the classical picture of a tumor.

Whether Chicken Tumor XVIII really metastasizes by means of cells cannot be determined from the data at hand. The general characters of the growth place it among the tumors and these, so far as we know, can be distributed only by cells. The tendency to localize in the muscles may be likened in its strangeness to the tendency of certain human neoplasms, for example, carcinoma of the prostate, to localize secondarily in the bones. In the case of Chicken Tumor XVIII the peculiarity attracts especial attention because of the many duplicate transplantation tumors that show it.

RELATION TO CHICKEN TUMOR I.

Though Chicken Tumor XVIII in its original form had few characters in common with Chicken Tumor I,—the first growth reported upon from this laboratory,—yet with repeated transplantation it has come to resemble it somewhat. Both are now spindle-celled sarcomata, with cells of medium size and very similar appearance. Both cause the host to waste away and die in coma. Filterable agents give rise to both. Chicken Tumor I was found in a Plymouth Rock fowl and its transplantation was for a long time most successful in fowls of this sort, while Chicken Tumor XVIII,

[13] Zahn, F. W., *Virchows Arch. f. path. Anat.*, 1889, cxvii, 1.

though occurring originally in a brown Leghorn chicken, has done strikingly well in Plymouth Rocks. It may properly be asked whether Chicken Tumor XVIII does not represent a form, originally much attenuated, of the disease which we know as Chicken Tumor I.

This does not seem to be the case. Despite a heightened malignancy which has rendered it of late more nearly comparable to Chicken Tumor I, Chicken Tumor XVIII has retained distinguishing characters, as can well be seen when both tumors are growing in one host at the same rate. Chicken Tumor XVIII is then observed to be a growth of fairly homogenous structure, firm from much collagen, and rarely necrotizing or becoming hemorrhagic; Chicken Tumor I is soft, and has a structure much varied by hemorrhage, necrosis, and mucoid degeneration. The giant cells and other degenerative forms which are a striking feature of Chicken Tumor I, are not found in Chicken Tumor XVIII. In certain brown Leghorn fowls in which both metastasized, Chicken Tumor I did so to the lungs and further viscera, while Chicken Tumor XVIII localized in the muscles and there only. When Chicken Tumor I is growing slowly in partially resistant hosts it does not exhibit the characters of Chicken Tumor XVIII in the earlier slowly growing generations, but keeps its own peculiarities. Finally, artificial attenuation of the agent causing Chicken Tumor I, by heating, or glycerinization, or storage after drying, has never so altered it that it will produce tumors resembling Chicken Tumor XVIII. The agents causing the two growths may nevertheless have some relationship to each other. The point remains to be investigated. But certainly the growths to which they give rise are separate entities.

SUMMARY.

A spontaneous chicken sarcoma, peculiarly fissured by blood sinuses, and with a tendency to intracanalicular extension into them, has been transplanted and studied in eight successive groups of fowls. Histologically the growth is a characteristic neoplasm, while in its transfer to new hosts a real transplantation is obviously involved. The development of the first few series of transplantation tumors was very slow. They exhibited the histological struc-

ture of the original growth and had the same tendency to metastasize to the skeletal muscles. Recently the tumor has grown more rapidly and in a higher percentage of hosts. With this has come a simplification of structure to that of a pure, spindle-celled sarcoma.

Fowls of an alien variety (Plymouth Rock) form quite as good hosts for the tumor as those of the sort (brown Leghorn) in which it was originally found. It has not grown in pigeons, rats, or mice.

The question of the cause of the tumor is not taken up in the present paper. It has been found to be due to an agent which will pass through Berkefeld filters. The growth is quite distinct in its characters from the other two transplantable neoplasms of the fowl (a spindle-celled sarcoma, an osteochondrosarcoma) which have such a cause. No growth like it has been observed among the forty-three spontaneous tumors of the fowl that have come under our observation.

EXPLANATION OF PLATES.[14]

Plate 49.

Fig. 1. Chicken Tumor XVIII in the gizzard of the original fowl. Three metastases are visible in the skeletal muscles, namely one in the neck, another in the thoracic wall above the right lobe of the liver, and the third on the inner surface of the pelvis. All are indicated by arrows.

Plate 50.

Fig. 2. Sagittal section through the gizzard and the primary growth.

Fig. 3. Sagittal section of the left leg of the original fowl to show the tumor masses in the muscles above and below the knee. Nearly all of the non-neoplastic tissue has been cut away. The extension of the growth into the joint cavity of the knee can be plainly seen.

Plate 51.

Fig. 4. Margin of the growth in the gizzard of the original fowl. To the left are normal muscle and the mucous membrane of the gizzard cavity. The dilatation of blood spaces that precedes the invasion of the growth should be noted, and the general absence of a cellular reaction. Many of the growth's subdivisions show an interstitial hydropic change, and at the extreme right its tissue is necrotic.

Fig. 5. Section through the tumor mass in the leg of the original host, to show the fissured character of the growth. The darkly stained bodies in the vessels are the nucleated red cells of the fowl.

[14] All the microscopic sections were stained with eosin and methylene-blue.

PLATE 52.

FIG. 6. Chicken Tumor XVIII in a fowl of the second generation, dying five and one half months after inoculation. The primary masses lie in the pectoral muscle to either side of the sternum. There are large secondary tumors in the muscles of both legs and in one wing. A small tumor nodule overlies the first joint of the other wing. The emaciation of the fowl is largely concealed by the growths.

FIG. 7. Section of the tumor in a fowl of the third generation, series A. The heavy black dots scattered here and there are artefacts. The fissured, intracanalicular structure is well shown.

PLATE 53.

FIG. 8. A metastasis in the lung. The growth is here a pure, spindle-celled sarcoma. The fissured primary tumor is shown in figure 7.

FIG. 9. Chicken Tumor XVIII replacing striped muscle. The muscle fibers are split up by the growth into numerous small fragments.

PLATES 54 AND 55.

FIG. 10. Section of another part of the same tumor, showing focal, hydropic degeneration. In the undegenerated area collagen fibrils are numerous.

FIGS. 11, 12, and 13. Three grafts of Chicken Tumor XVIII shown in cross-section with the surrounding tissue. All were implanted at the same time in a susceptible, normal fowl and removed one, two, and three days later, respectively. They lie just beneath the epidermis in the pars reticularis of the corium.

The graft removed one day after implantation is unattached and its cells are living except toward the center of the fragment where necrosis is already apparent. There is some cellular reaction about the tumor bit removed at the end of the second day. Though as yet unvascularized, it is here and there attached to the host tissue, and most of its cells are living. At the end of the third day vascularization has occurred, and the tumor cells, successfully transplanted, are proliferating and extending into the host tissue.

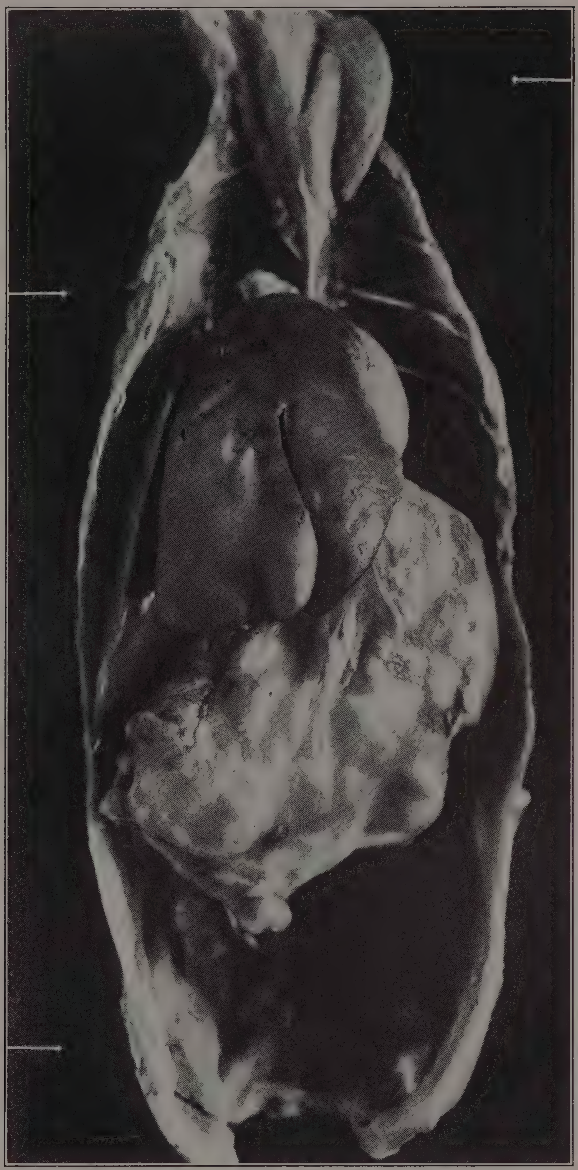


Fig. 1.

(Rous and Lange: Chicken Tumor Due to Filterable Cause.)

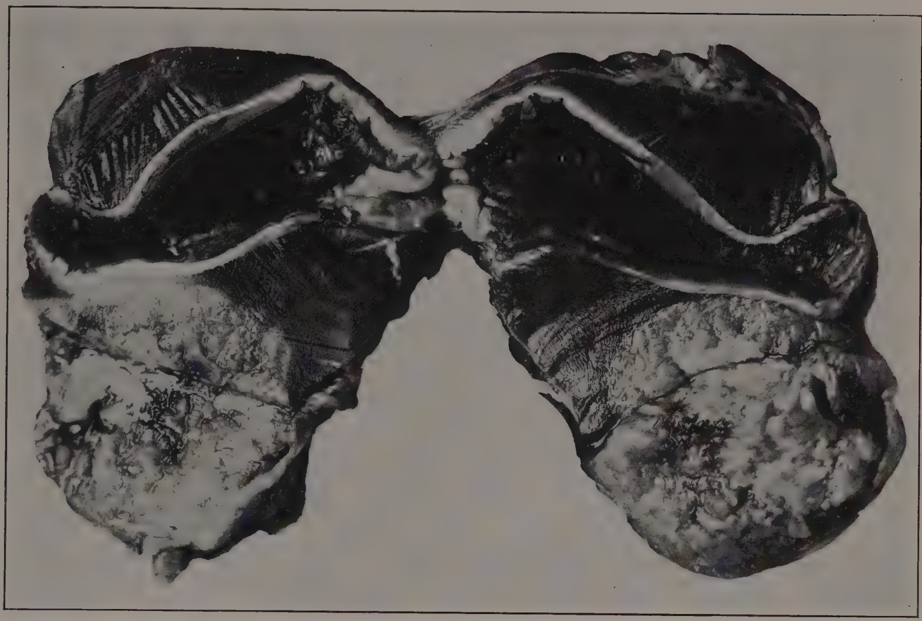


Fig. 2.

Fig. 3.

(Rous and Lange: Chicken Tumor Due to Filterable Cause.)

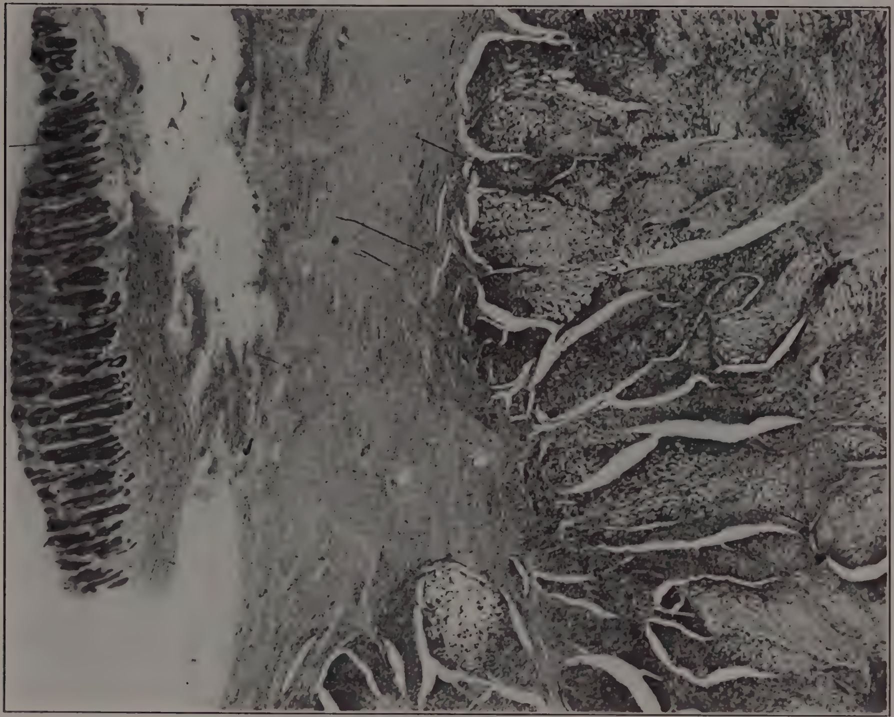


Fig. 4.

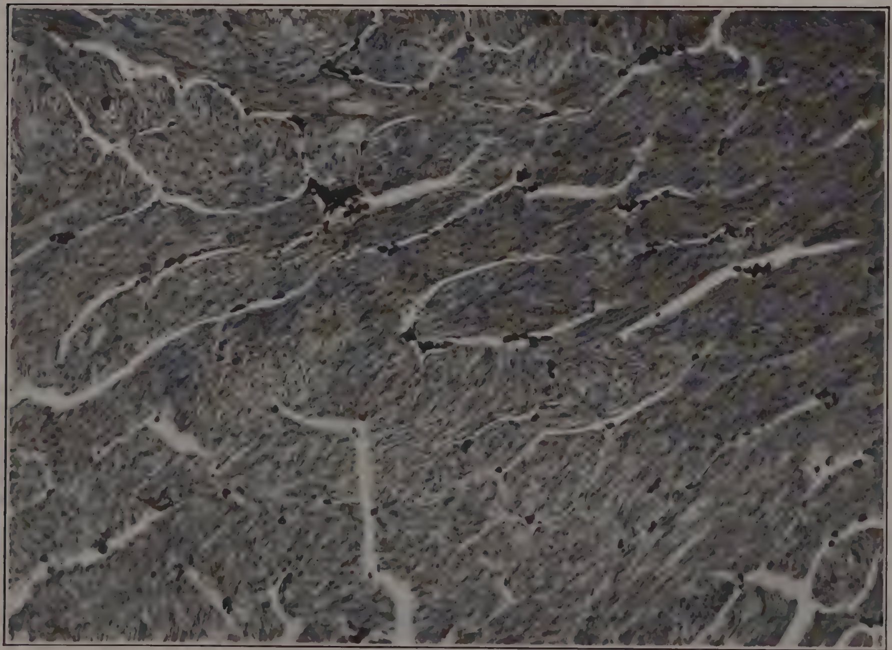


Fig. 5.

(Rous and Lange: Chicken Tumor Due to Filterable Cause.)

Fig. 6.

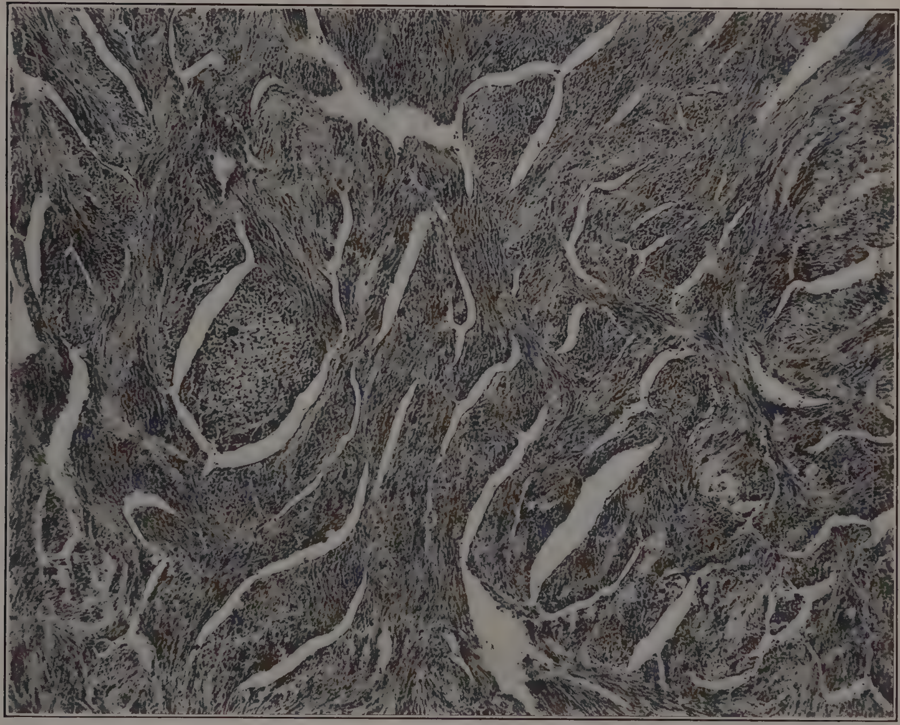


Fig. 7.

(Rous and Lange: Chicken Tumor Due to Filterable Cause.)

FIG. 8.

FIG. 9.

(Rous and Lange: Chicken Tumor Due to Filterable Cause.)

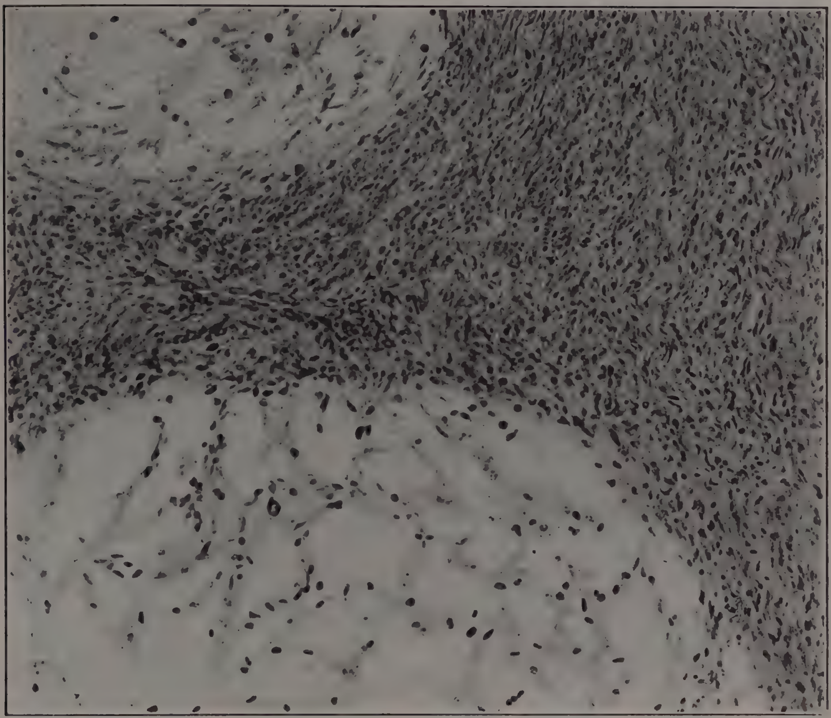


Fig. 10.

Fig. 11.

(Rous and Lange: Chicken Tumor Due to Filterable Cause.)

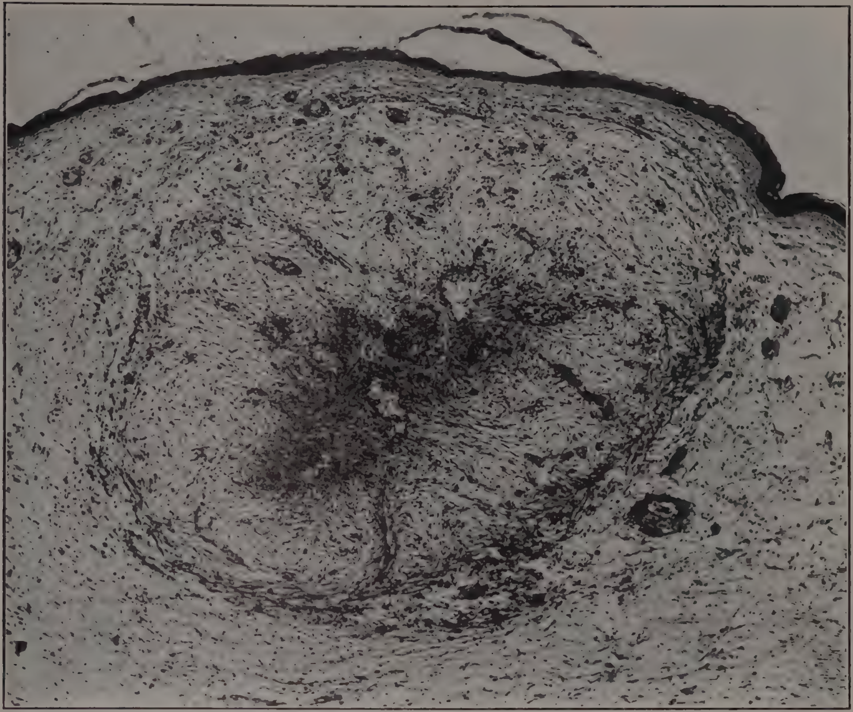


Fig. 12.

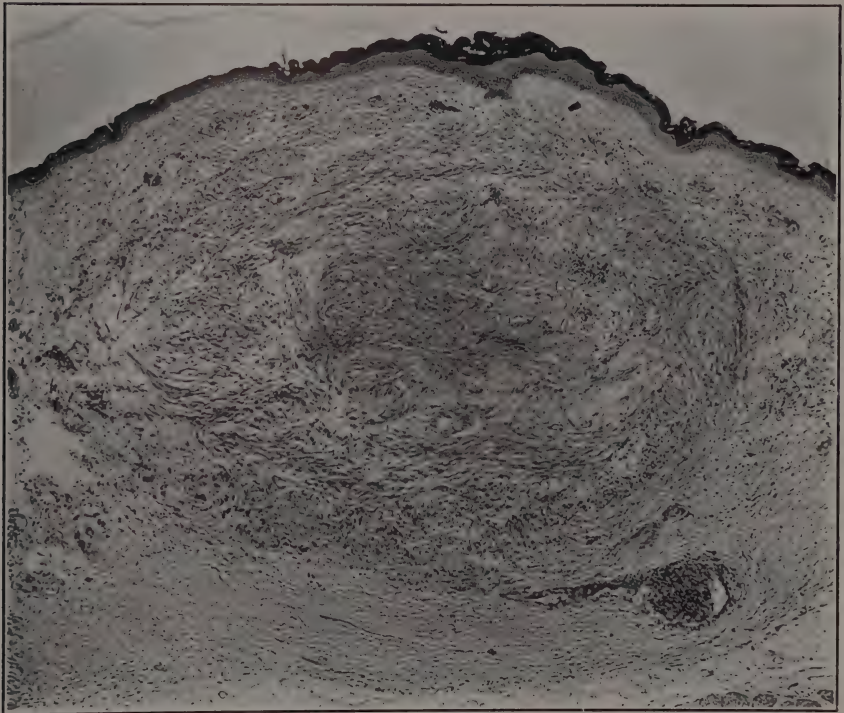


Fig. 13.

(Rous and Lange: Chicken Tumor Due to Filterable Cause.)

ON THE CAUSATION BY FILTERABLE AGENTS OF THREE DISTINCT CHICKEN TUMORS.

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ON THE CAUSATION BY FILTERABLE AGENTS OF THREE DISTINCT CHICKEN TUMORS.*

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PLATES 7 TO 12.

The study of chicken tumors has especial interest because the cause of two such growths has been found in filterable entities. The object of the present paper is to report experiments which show that yet a third neoplasm of the fowl is so caused, and to discuss at some length the methods and findings with all three. One of the growths,—Chicken Tumor I in our series of spontaneous chicken tumors,—is a pure spindle-celled sarcoma;[1] the second (Chicken Tumor VII) is an osteochondrosarcoma;[2] and the third (Chicken Tumor XVIII), of which the cause will here for the first time be reported, is a spindle-celled sarcoma of peculiar intracanalicular pattern.[3] The three are very unlike, not only histologically but in their general behavior. Yet, as will be seen, the entities causing them have much in common and may profitably be considered together.

THE IMPORTANCE OF PRELIMINARY TRANSPLANTATION.

In our experience the transplantation of chicken tumors is of great importance for experiments looking to their cause. This is in part on account of the material afforded by successful transplantation, but it has its essential basis in the enhanced malignancy resulting therefrom. The findings have shown strikingly that the more malignant the growth the easier is the demonstration of its etiological agent. The fact that tumors caused by an agent dam-

* Received for publication, October 15, 1913.

[1] Rous, P., *Jour. Am. Med. Assn.*, 1911, lvi, 198; *Jour. Exper. Med.*, 1911, xiii, 397. For the literature see Rous, P., and Murphy, J. B., *Berl. klin. Wchnschr.*, 1913, l, 637.

[2] Rous, P., Murphy, J. B., and Tytler, W. H., *Jour. Am. Med. Assn.*, 1912, lix, 1793. Tytler, W. H., *Jour. Exper. Med.*, 1913, xvii, 466.

[3] Rous, P., and Lange, L. B., *Jour. Exper. Med.*, 1913, xviii, 651.

aged in some way, as for example by heat or by long sojourn in the dry state, are of relatively benign course[4] suggests that the ready separation of the agent from growths of high malignancy is dependent on its enhanced activity.[5]

THE TUMOR-PRODUCING ENTITIES ARE FILTERABLE.

Three methods, namely filtration, desiccation, and glycerination, have been chiefly used to demonstrate a cause for the chicken tumors, as distinct from the tumor cells. Specimen protocols will be given to illustrate the results with each method and these will be followed by a summary of the findings obtained by its employment. The growths produced have in every instance been examined with the microscope.

Filtration has proved by far the most uniformly successful method of study. Under suitable conditions the agents of all the growths will pass through Berkefeld filters that retain small bacteria (*Bacillus prodigiosus, Bacillus fluorescens liquefaciens*). Our technique has been described several times. It is again briefly given in the first of the two following protocols that show the filterability of the agent causing Chicken Tumor XVIII.

Chicken Tumor XVIII, a Sarcoma of Intracanalicular Pattern. Filtration Experiment 1.—The tumor material came from fowl 257 of the second transplantation generation, series A. Fresh neoplastic tissue to the amount of 7.5 gm. was ground fine with sand, taken up in 250 c.c. of Ringer's solution at 40° C. and a 24 hour culture on slant agar of *Bacillus fluorescens liquefaciens* was added. Shaking was done in a machine for forty-five minutes, then brief centrifugalization, and portions of the supernatant fluid were filtered by suction through one or another of three Berkefeld filters; (a) a medium sized V (No. 3), and (b) and (c) two small N cylinders (No. 5). Filtration was continued for about thirty minutes. The fluids from filters a and b were united as filtrate AB. That from filter c will be called filtrate C. To each was added a little sterile diatomaceous earth (*Kieselguhr*) and then 14 c.c. of AB and 7 c.c. of C were injected intramuscularly in the right and left pectoral regions respectively of five normal brown Leghorn fowls. All remained free of tumor until 170 days after the inoculation, when in one a small mass, 2.5 by 1.5 cm. in diameter was noted at the site of injection of filtrate AB. Part of this was removed and found to have

[4] Rous, P., and Murphy, J. B., *Jour. Exper. Med.*, 1913, xvii, 219.

[5] It may not be amiss to point out that in epidemic poliomyelitis, a disease caused by a filterable virus or parasite, the initial infection of monkeys with human nervous tissues is accomplished with difficulty, while after the virus has

the characteristic structure of Chicken Tumor XVIII. The fowl was shortly afterwards lost through accident. The other chickens were kept some months longer but never developed tumors.

Plates of the filtrates (1 c.c. to the tube of agar) remained sterile, whereas plates of the unfiltered fluid (a few drops to the tube of agar) showed innumerable colonies of *Bacillus fluorescens liquefaciens*. At the time of the experiment bits of the tumor which furnished material for it were transplanted into three chickens. Two of these developed tumors within a month. The third remained healthy.

Filtration Experiment 2.—The material was furnished by fowl 531 of the third transplantation generation, series C. The tumor was of especially rapid growth. Three Berkefeld filters were employed: (a) a medium sized V cylinder (No. 3), and (b) and (c) two small N cylinders (No. 5). *Bacillus fluorescens liquefaciens* was used as before; and the filters proved impermeable to it. To each of the filtrates, A, B, and C, a little finely ground, sterile, diatomaceous earth was added previous to their separate injection in amounts of 5 c.c. each, into the muscle of the lower leg and breast of eight fowls. Two of the fowls developed progressively growing tumors which appeared, in the one case at sites A and C two months after the time of injection, in the other at sites A and C about five months after it. Both died of the tumor some seven and one half months after inoculation. In addition to the large primary growths there were metastases in the lungs, and in one case secondary growths about several joints (figure 1), a finding not infrequent when Chicken Tumor XVIII is propagated by transplantation. The hip joint was diffusely involved in sarcomatous tissue and fusiform swellings enclosed most of the sternal and vertebral ribs at their junction, forming what might be called a sarcomatous rosary. Whether in the present case these represent primary localizations of the tumor-producing virus or true metastases cannot be said. Microscopically all the growths had the characteristic structure of Chicken Tumor XVIII (figure 2).

In a third fowl a tumor nodule appeared within two months at the site of filtrate B, slowly enlarged to a diameter of 4 cm., and slowly retrogressed again. The only transplantations attempted from the filtrate tumors were performed with this growth. They gave negative results. The other fowls injected with filtrate remained healthy.

The association of a foreign body with the filtrate to bring about a tissue derangement renders much more likely the production of tumors. The influence of the factor has been carefully studied in the case of Chicken Tumor I.[6] In all the experiments we have made use of powdered diatomaceous earth (*Kieselguhr*), which elicits, as Podwyssozki has shown, an intense reactive connective tissue proliferation with the formation of giant cells. Histologically this

been transmitted through several monkeys, it increases markedly in virulence for those animals and can be transferred with far greater certainty.

[6] Rous, P., Murphy, J. B., and Tytler, W. H., *Jour. Am. Med. Assn.*, 1912, lviii, 1751.

reactive proliferation bears no resemblance to the tumors with which we have worked (figure 10).

Chicken Tumor XVIII. Filtration Experiment 3.—Filtrates were prepared in the usual way, through filters which the control with *Bacillus fluorescens liquefaciens* showed to be bacteria-tight. Three Berkefeld cylinders were used: (a) a large unmarked cylinder (No. 2), (b) a medium V (No. 3), and (c) a small N (No. 5). The filtrates were separately examined for the test bacterium, and injected. To half of each was added enough sterile, powdered *Kieselguhr* to give a slight cloudiness. For this very little was required. Six fowls were injected, receiving on one side in the thigh and pectoral muscle respectively two filtrates with *Kieselguhr,* at corresponding points on the other side the same filtrates in equal or slightly greater amount, without *Kieselguhr.* Of the five fowls that survived three months one developed after the sixtieth day small tumor nodules, where the filtrates A and C had been injected in amounts of 10 c.c. and 3 c.c. respectively with *Kieselguhr.* The corresponding sites, where slightly larger quantities of the filtrates without admixture had been put, remained free of growths. The tumors measured 4 cm. and 3 cm. in diameter respectively when the fowl was killed three months after injection. They had the characters of Chicken Tumor XVIII. The other fowls, kept long under observation, remained healthy.

Chicken Tumor VII, an Osteochondrosarcoma. Filtration Experiment 2.—Six normal fowls were injected in the pectoral muscle on the right side with 6 c.c. each of an active Berkefeld filtrate, in the right thigh muscles with 4 c.c. of the same filtrate to which had been added a little *Kieselguhr.* It has been repeatedly found that the thigh muscles furnish a relatively poor site of inoculation. Nevertheless, in four of the fowls rapidly growing cartilaginous tumors of multicentric origin appeared in the thigh (figures 3 to 8), whereas a pectoral tumor developed in only two of the four. It took the form of a discrete cartilaginous nodule in the track of the injecting needle. The other two fowls remained healthy. One of the tumor fowls furnished material for the experiment which follows and also for a successful attempt to dry the etiological agent. Some of the growths contained so much bone that the saw was required to lay them open.

Filtration Experiment 4.—Portions of a tumor extract prepared as usual and with the usual bacterial control were passed through one of the following Berkefeld cylinders: (a) and (b) two small N cylinders (No. 5), and (c) a medium sized V (No. 3). The tumor material came from one of the fowls of filtration experiment 2. Filtrates A and B were injected separately in amounts of 10 c.c. into the leg muscles of three normal fowls. Filtrate C with and without admixture of *Kieselguhr* was injected into the pectoral regions of the same fowls. Nineteen days later one had developed a tumor nodule 1.3 cm. in diameter at the spot where 5 c.c. of filtrate C with *Kieselguhr* had been injected. The nodule rapidly enlarged to a diffuse mass which led to the death of the fowl after six weeks in all. At this time a nodule had just appeared in the other pectoral region where 10 c.c. of filtrate C without admixture had been put. At autopsy another small discrete nodule was found in the left leg as the result of 10 c.c. of filtrate A without *Kieselguhr.* The other two fowls remained healthy.

Chicken Tumor I, a Spindle-Celled Sarcoma. Injury Experiment 15.—An active Berkefeld filtrate was prepared as usual and to half of it a little *Kieselguhr* was added. 9 c.c. of the mixture was injected into the pectoral muscles on one side of eight fowls, and on the other as control an equal amount of the plain filtrate. The results are shown in text-figure 1. It will be seen that where there was *Kieselguhr* tumors arose much more rapidly, and when palpable were already diffuse. In this instance nearly all the fowls responding with a tumor to the filtrate plus *Kieselguhr* eventually developed growths where the filtrate alone had been placed. Ordinarily this happens in only a small percentage.

SUMMARY ON FILTRATION.

The growths engendered by a Berkefeld filtrate have the distinctive characters of the strain of tumor which furnished the material for filtration (figures 1 to 9). In the case of Chicken Tumors I and VII growths so caused have themselves been successfully used for the preparation of filtrates, as for example in one of the experiments just cited. They have also been found to be transplantable. The results with Chicken Tumor XVIII are relatively meagre owing to the long period of latency after the injections,—more than five months in one case,—and to the slow growth of the filtrate tumors. The agents causing Chicken Tumors I and VII often pass the filter in large amount, as shown by the multicentric development of tumors in the region injected, but even when the element of tissue derangement is present, Chicken Tumor XVIII usually appears from one, or at most a few, centers. This is more probably due to low average virulence or resistance in the agent than to difficulties in filtration, for the findings show that all the agents pass or are held back by filters of about the same texture. They pass most V cylinders, many designated as N, but they are usually retained by the fine textured W cylinders. As might be expected from these results the agent of Chicken Tumor I,—the only one thus tested so far—fails to pass Chamberland bougies.

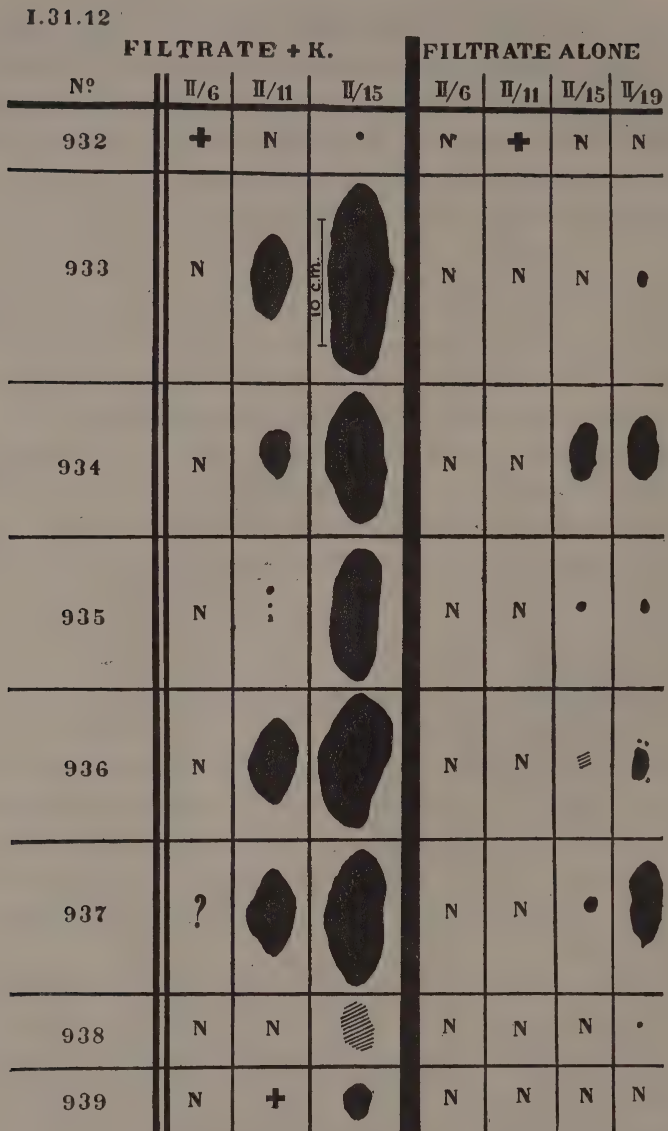
Not infrequently filtrates prepared from malignant material under the best conditions prove entirely innocuous. This is in most cases due to the narrow limits within which the agents are filterable. Two possibilities suggest themselves as accounting for these limits, first that the agents are formed bodies, second that if unformed they are associated with substances which clog the pores of the filters. Mucinous substances are so abundant in Chicken Tumor I

that coarse filters are soon completely stopped by very dilute extracts of the growth; but with VII and XVIII there is no such complication. Extracts of these tumors run rapidly through the filters and yet are often inactive. The agents then would seem to be of relatively large size among the filterable causes of disease.

The tumor resulting from a local injection of filtrate appears at the site of inoculation. As already mentioned the importance of tissue derangement for the action of all three causative agents is very great. The experiments show that when this factor is supplied by the addition of *Kieselguhr* to a filtrate the percentage of fowls that develop tumors is much increased and the growths themselves appear sooner and enlarge more rapidly (text-figure 1). The *Kieselguhr,* injected alone, does not cause tumors. A limpid filtrate injected in the breast muscle finds its point of action in the track of the injecting needle (figure 9), and there results a discrete growth from one center. Oftentimes, as ordinarily prepared, the filtrate contains a few particles from the interior of the filter, and the growth may then arise from several centers. But when powdered *Kieselguhr* has been added the growth is multicentric and appears all at once as a mass of coalescing foci. The microscopic findings show that the sarcomatous change in the reactive tissue about the *Kieselguhr* is not diffuse but punctate, and the growth of the little tumors is largely expansive (figure 10). In fowls inoculated intravenously with a filtrate of Chicken Tumor I the tumors have been found to arise at sites of tissue derangement.[7] Their incidence is approximately doubled when *Kieselguhr* has been introduced into the blood stream.

As already mentioned the reactive tissue called forth by *Kieselguhr* contains large numbers of giant cells. These are of ordinary foreign body type and enclose the *Kieselguhr* fragments. With the replacement of the reactive tissue by tumor the giant cells are destroyed (figure 10) and the *Kieselguhr* is set free. It can be found here and there among the sarcoma cells, which appear in no way affected by it.

[7] Rous, P., Murphy, J. B., and Tytler, W. H., *Jour. Am. Med. Assn.,* 1912, lviii, 1751.



TEXT-FIG. 1. CHICKEN TUMOR I. INJURY EXPERIMENT 15. Eight normal Plymouth Rock fowls, Nos. 932 to 939, were injected in one pectoral region with a Berkefeld filtrate of Chicken Tumor I, in the other with an equal quantity of the same filtrate to which had been added a little powdered diatomaceous earth (*Kieselguhr*). Examinations were made every week thereafter and the size of the tumors charted. It will be seen that where filtrate plus *Kieselguhr* was injected tumors appeared more frequently and much earlier, and were diffuse when first noted in sharp contrast to the discrete nodules on the other side.

DESICCATION.

Since many microörganisms withstand drying and the tissue cells of the higher animals do not, workers have frequently used desiccation in attempts to separate an etiological agent from mammalian tumors. The literature need not be discussed since it deals only with negative results. The agents causing two of our chicken tumors, namely the pure spindle-celled sarcoma (Chicken Tumor I), and the osteochondrosarcoma (Chicken Tumor VII), resist drying. No special complications are met with in obtaining an active dry form of the agent of Chicken Tumor I, but in the case of Chicken Tumor VII successful results have been obtained only by the desiccation of frozen material. A few specimen protocols will be given.

Chicken Tumor I. Desiccation Experiment 2.—The tumor material, obtained from fowl 243 of the ninth transplantation generation, series A, was ground fine and spread thin in a desiccator containing sulphuric acid. A partial vacuum only was produced. After forty-eight hours the scales of dry tissue were pulverized in a mortar and replaced in the desiccator. After sixty-seven hours in all the powder was taken up in Ringer's solution by grinding, in the proportion of 2 gm. to 70 c.c. 5 c.c. of the turbid, viscous fluid were injected into each breast of seven normal hens. Six showed rapidly growing tumors at the end of the month. The seventh remained healthy. One of the fowls furnished tumor material for the experiment which follows.

Desiccation Experiment. 4.—A fowl of experiment 2, with a large tumor, was killed and the neoplastic material dried as before, for forty-eight hours; ground; replaced in the desiccator for seventy-two hours more; and then sealed in glass and kept in the dark at about 2° C. Fifty-four days later 2 gm. of the material were taken up in 25 c.c. of Ringer's solution, and the thick suspension was inoculated in amounts of 5 c.c. into each breast of five normal hens. Three of these had developed tumors two weeks later, and one growth was already large. In a fourth fowl the tumor appeared later. The fifth remained quite healthy.

Some of the dried material was left sealed and in the cold for seven months. 3 gm. of it were then taken up in 20 c.c. of normal salt solution and 1 c.c. of the mixture was injected into one breast of eight fowls. Of these two had developed small tumors after forty days. The others remained healthy. The growths were typical Chicken Tumor I.

Chicken Tumor VII. Desiccation Experiment 3.—Fowl 549, carrying a large growth engendered by a filtrate (filtration experiment 2) was killed, and the tumor, which consisted of cartilage and precartilaginous, sarcomatous tissue, was ground fine and placed in a cold compartment at several degrees below 0° C., where in the course of forty-eight hours it gradually froze solid. While still frozen it was put in a cold desiccator over sulphuric acid, and the air exhausted until the manometer showed a pressure of less than 1 mm. of mercury. The desiccator was then placed immediately in an ice chest. At the end of three days the material was taken out and ground. It had the form of a light, brittle cake

of nearly the bulk of the fresh tissue, was cottony in texture, and ground with great difficulty. Twenty-four hours after grinding, 2 gm. of the powder were taken up in 25 c.c. of sterile water and of the thick fluid 5 c.c. were injected into the left breast of each of five normal fowls. Within a month one developed a growth measuring 4 by 9 cm. It was killed and portions of the tumor were used for the experiment that follows. The tumor consisted of a spindle-celled sarcomatous tissue undergoing cartilaginous differentiation in the way characteristic of Chicken Tumor VII. The other fowls, kept under observation several months, remained healthy.

Desiccation Experiment 4.—The material came from the susceptible fowl of the previous experiment. It was ground, frozen, and dried while still frozen, by the method just outlined. It remained in the freezing chamber three days, in the desiccator twenty-four hours, and was then powdered and placed in the ice chest for twenty-four hours more. Eight fowls were injected in the left breast with 5 c.c. each of a thick fluid made by taking up 2 gm. of the powder in 40 c.c. of distilled water. In the other breast glycerinized tumor tissue was placed at the same time (glycerin experiment 2). Within three weeks all except one of the injected fowls had developed a tumor mass at the site of inoculation of the dried material, the largest measuring 3.4 by 7.5 cm. Four of the fowls were allowed to die of their growth, the other three being killed and examined at various times. All the growths presented the character of Chicken Tumor VII, with much cartilage and in several instances bone. The eighth fowl remained healthy.

In this experiment there was the complicating factor of tumors developing synchronously from glycerinated material. But that the tumors which arose where dried material had been injected were engendered by this is certain, not only from the local character of the neoplastic disease in its early stages, but from many other instances that might be cited in which the dried tissue of Chicken Tumor VII has been successfully used to cause the growth.

SUMMARY ON DESICCATION.

The growths caused by dried tissue of Chicken Tumors I and VII appear at the site of injection and themselves furnish material which has a tumor-producing activity when dried. The etiological agent of Chicken Tumor I undergoes a gradual attenuation when the dried tissue is stored in the dark at a temperature slightly above 0° C., but after seven months is still capable of producing tumors. In the case of Chicken Tumor VII no attempt has been made to store dried material for longer than three weeks. At the end of this time it still produced tumors.

Numerous attempts have been made to obtain the agent of Chicken

Tumor XVIII in an active dry form, but as yet without success. In this connection it should be recalled that Chicken Tumor XVIII under the best conditions grows much more slowly than I and VII and that a filtrate of it gives rise only exceptionally to tumors and after a long latent period. The agents of Chicken Tumors I and VII when dried at low temperature are both very active; but the one easily survives drying at room temperature whereas the other fails to. Differences in the viability of organisms would well account for this.

The growths caused by dried material spring from multiple foci and are often diffuse when first palpable. The bits of dead tissue doubtless act to produce like *Kieselguhr* a tissue derangement favorable to the production of tumors.

GLYCERINATION.

The effect of glycerin has especial interest because of the ultramicroscopic organisms which retain their vitality when immersed in it. Loeb[8] has observed its effect upon mammalian tumor tissue. He placed pieces of rat sarcoma in pure glycerin, and, seventeen to twenty-four hours later, washed them in salt solution and injected bits into other rats. In some instances a tumor developed. If it can be assumed that the glycerin thoroughly penetrated the tumor tissue the finding is significant. For this reason we have repeated and enlarged upon Loeb's experiment, previous to observations with the chicken tumors. Briefly, the findings show that pieces of the Jensen rat sarcoma 0.5 cm. thick, when kept for twenty-four hours in pure glycerin at a temperature of 1° to 2° C., will sometimes give rise to tumors on being cut up and implanted; but the same material, finely chopped and stirred into the glycerin, yields only negative results, even when well washed and injected in quantity. Rat sarcoma treated according to the latter method occasionally retains its viability for twenty-four hours in a 12.5 or 25 per cent. glycerin mixture with Ringer's solution, but never in 50 per cent. glycerin, nor for a longer period (four days) in the weak dilutions mentioned. Hence it seems then that the positive results with pieces of rat tumor kept in concentrated glycerin are dependent on incomplete penetration.

[8] Loeb, L., *Virchows Arch. f. path. Anat.*, 1903, clxxii, 345.

Of the filterable entities that cause chicken tumors the two which withstand drying will also survive glycerination.

Chicken Tumor I. Glycerin Experiment 3.—13 gm. of fresh tumor tissue from fowl 582 (16th generation B) were ground with sterile sand, and suspended in 40 c.c. of Ringer's solution. The larger tissue particles were got rid of by straining, and the sand was allowed to settle out. Of the thick suspension of tissue 10 c.c. were stirred into an equal amount of pure glycerin, shaken until well mixed and placed in the ice box. After seven days the tissue fragments, which had by this time settled, were drawn off through the base of the tube by breaking a sterile glass projection in its wall. In this way all contamination with particles which had escaped the glycerin's action was avoided. The tissue suspension was now washed with Ringer's solution, centrifugalized, and of the pasty sediment 0.15 c.c. was injected into the right and left pectoral muscles respectively of three young Plymouth Rock fowls. Ten days later in one fowl a small sarcomatous nodule made its appearance at each injection site, and in a second after seventeen days a single nodule developed. The subsequent growth of the tumors was slow, but otherwise they proved characteristic of Chicken Tumor I. The third fowl remained healthy.

Glycerin Experiment 2.—Fresh tumor tissue was ground in a mortar with sand and taken up in Ringer's solution. By rapid centrifugalization the suspension was rid of all but extremely minute tumor fragments. To portions of it pure glycerin was added to the amount of 50, 25, and 12 per cent. respectively of the total bulk. The mixtures were placed in wide tubes and kept in the dark at 1° to 2° C. After nine days some of the material was withdrawn by breaking a projection at the base of the tube, as in the experiment already described. Twenty-two days later a fowl injected in each pectoral region with 0.4 c.c. of the mixture in 50 per cent. glycerin, diluted to 4 c.c. with Ringer's fluid, had developed very large tumors having the character of Chicken Tumor I. In the three fowls injected with 12 and 25 per cent. mixtures, variously diluted, growths had also appeared at this time.

Some tubes of the 12.5 and 25 per cent. mixtures were left untouched and in the cold for thirty-one days. Their contents were then drawn off as usual. One fowl injected in each breast with 3 c.c. of 25 per cent. mixture, undiluted, developed tumors. These first became palpable after six weeks. Another fowl injected in the same way with 1 c.c. of the 25 per cent. mixture made up to 2 c.c. with Ringer's fluid likewise slowly developed tumors. Three fowls inoculated with the 12.5 per cent. mixture, diluted with an equal bulk of Ringer's solution, remained healthy.

Higher percentages of glycerin have been used in the experiments with Chicken Tumor VII.

Chicken Tumor VII. Glycerin Experiment 3.—Cartilaginous tumor material was ground to a foamy pulp in a meat chopper and two mixtures were made with pure glycerin, in the amounts of (a) 2 c.c. tumor pulp and 8 c.c. glycerin, and (b) 4 c.c. tumor pulp and 10 c.c. glycerin. The actual quantities of tumor tissue were considerably less than those mentioned, as it had the form of a

soufflé. The mixtures were tubed and kept in the cold as usual. Sedimentation in the glycerin was extremely slow and shaking was done each day to keep the larger tissue fragments in suspension. After thirteen days the tubes were opened at the base, and the mixtures, made up to 20 c.c. with Ringer's solution, were injected in amounts of 5 c.c. into the right and left pectoral region, respectively, of three normal fowls. After two months one fowl developed a tumor that in another three months proved fatal. The growth was 6 cm. in diameter at this time and in addition to much cartilage contained bone. It had arisen at the site of injection of the mixture that contained the greater percentage of glycerin (8 c.c. to 2 c.c. of tumor). The other fowls remained healthy.

Glycerin Experiment 4.—Fresh cartilaginous tumor material was ground to a soufflé and three mixtures were made: (a) 10 c.c. of pure glycerin with an equal bulk of tumor material, (b) 10 c.c. of glycerin with 5 c.c. of tumor, and (c) 10 c.c. with 3 c.c. of tumor. These were kept in the ice box; shaken every day; at the end of ten days drawn off from below; and each made up to 20 c.c. with Ringer's solution and inoculated in amounts of 5 c.c. in one pectoral region of five normal fowls. Four of these died of intercurrent disease. Ninety-nine days after the injection the fifth had developed a tumor measuring 8 cm. at the inoculation site of mixture (c), and one of half this size where mixture (a) had been put. Both growths were characteristic of Chicken Tumor VII and both contained bone.

SUMMARY ON GLYCERINATION.

The experiments leave no doubt that the tumor tissue was thoroughly penetrated by glycerin. The agent of Chicken Tumor I retains some activity for at least seven days in 50 per cent. and for thirty-one days in 25 per cent. glycerin. The effect upon it of higher concentrations has not been tested. In the case of Chicken Tumor VII the amount of glycerin has ranged from 50 to about 90 per cent., but no attempt has been made to determine the period of survival of the agent. That it remains active in the high concentrations for at least thirteen days is shown by experiment 3. Glycerination undoubtedly has an attenuating action on both agents. The tumors develop in few hosts, and after a relatively long latent period, and often grow slowly. It has been repeatedly noted that the activity of the agents is best retained in high concentrations of glycerin. This might be thought due to differences in penetration of the tumor tissue attendant upon differences in concentration, were it not for the findings with rat sarcoma. Here concentrated glycerin proves the more injurious. Tissue autolysis in the dilute glycerin mixtures affords a better explanation of the results with the avian tumors. The most active agent, that of Chicken Tumor I,

has been found to be quickly destroyed by autolysis of the tumor tissue.

Repeated attempts to preserve in glycerin the agent causing Chicken Tumor XVIII have been unsuccessful.

SEPARATION OF THE AGENTS BY OTHER METHODS.

Only in the case of Chicken Tumor I have still other attempts been made to distinguish the tumor-producing agent from the neoplastic cells. Ultraviolet rays kill the cells and leave the agent unharmed.[9] The method requires careful control and is not available for the separation of large amounts of the agent. The resistance of the agent to heat is only very slightly greater than that of the cells.[10] On the other hand, the agent withstands freezing and thawing which reduce the associated tumor tissue to a pulp.

DISCUSSION.

In the first attempts to isolate a causative agent from Chicken Tumors VII and XVIII exact precautions were taken to avoid a possible contamination with the agent of Chicken Tumor I, though such contamination had never been observed in the many routine transplantations of these growths. To avoid exposure of the material to the laboratory air it was ground in a large sterile box and otherwise protected. The results of the experiments showed that these precautions were unnecessary, for the character of the tumors engendered by the agents effectually proved that they were not the result of contamination. Each agent produces only growths of the kind from which it came. One stimulates connective tissue to proliferate and elaborate cartilage, ultimately to be replaced in greater or less part by bone (Chicken Tumor VII); another causes connective tissue to proliferate and form large undifferentiated spindle-celled masses (Chicken Tumor I); while a third engenders, like the second, a spindle-celled growth, but one containing much collagen, and characteristically fissured by blood sinuses, into which the growth shows a tendency to extend, resulting in a complex intracanalicular pattern (Chicken Tumor XVIII). The behavior of the

[9] Rous, P., *Jour. Exper. Med.*, 1913, xviii, 416.

[10] Rous, P., and Murphy, J. B., *Jour. Am. Med. Assn.*, 1912, lviii, 1938.

several growths is as different as is their histology. Chicken Tumor I metastasizes to the lungs by preference, then to the further viscera; Chicken Tumor VII almost never gives metastases; and Chicken Tumor XVIII frequently disseminates to the muscles without other secondary localization. The individuality of the agents as exemplified in the neoplasms that they cause is not altered by attenuating them, yet it should be mentioned that Chicken Tumor XVIII, the fissured sarcoma, has recently shown a tendency, like some complex mouse tumors, to lose with repeated transplantation its histological peculiarities and become an undifferentiated spindle-celled sarcoma. Ultimately the agent causing it may produce tumors not very different from Chicken Tumor I.

The tumors engendered by the filterable agents become palpable only after a latent period,—which in the case of Chicken Tumor XVIII is usually several months. Chicken Tumors I and VII appear more promptly. Fowls which fail to develop the growths within one month after an injection of the agent remain free of them, as a rule. When large amounts of filtrate plus *Kieselguhr,* or of dried or glycerinated material, have been injected the resulting growths are diffuse when first observed, owing to proliferation from many foci, and they quickly become massive; but the period of latency—at least eight to ten days in the case of Chicken Tumors I and VII—is not appreciably shortened. That the greater part of this period is one of actual latency with tumor cells absent as yet, and is not merely an interval during which the tumor is growing but clinically imperceptible, has been determined by the early microscopic examination of sites where the agent is known to be present and active.

The injection of large amounts of the agent of Chicken Tumor VII results in tumors that grow progressively and soon lead to the death of the fowl; whereas the growths developing after implantation of a small bit of the tumor enlarge slowly and in most fowls become stationary and eventually retrogress. This difference might be thought due to the influence of dosage as affecting resistance,—a factor of much importance in the case of mouse tumors,—or it might conceivably result from the circumstance that growths induced by an agent are elaborated by the host's own tissues, while

such as result from transplantation represent tissue growing in a host to which it is strange. That the latter explanation is not fanciful has been shown by experiments with Chicken Tumor I demonstrating the existence in fowls of two types of resistance directed, the one against the tumor-producing agent, the other against the transplanted tumor cells.[11] But in the case now being considered differences in dosage as affecting resistance are probably at the root of the matter. For by the injection of large amounts of the fresh tissue of Chicken Tumor VII in the form of a pulp, progressively growing tumors, such as result from massive doses of the agent, can be obtained.

The growths resulting from injection of a tumor-producing agent into the skeletal muscles are at first purely local in character, even when a filtrate is employed. In only one among many autopsies have growths been found which by their situation suggested a possible primary dissemination by the blood stream. This case is cited in one of the protocols (Chicken Tumor XVIII, filtration experiment 2). There is, of course, no reason why part of a filtrate, injected with a sharp needle, should not often pass directly into some blood vessel. But it has been shown with Chicken Tumor I that the direct intravenous inoculation of an active filtrate usually fails to produce tumors.[12]

Many fowls are resistant to the tumor-producing agents in any of the forms that we have used. Glycerination markedly reduces the agents' activity and desiccation does so to a less degree, and roughly in proportion to the length of time that the material is kept after drying. Both filtrates and dried tissue prepared under the best conditions from malignant material are sometimes unaccountably inactive. More often the inactivity can be traced to the use of too concentrated extracts for filtration, too finely textured a filter, or too slow a process of drying.

The findings with the three tumor-producing agents have a striking similarity and it is difficult to avoid the conclusion that the three are of one class, whatever that class may be. All give rise to dis-

[11] Rous, P., *Jour. Exper. Med.*, 1913, xviii, 416.

[12] Rous, P., Murphy, J. B., and Tytler, W. H., *Jour. Am. Med. Assn.*, 1912, lviii, 1751.

eases of neoplastic character, all act after a more or less pronounced latent period, the action of all depends to a striking extent on associated tissue derangement, and all pass through Berkefeld cylinders of about the same porosity, being held back by others of slightly finer grade. Two of them resist drying and can be preserved in glycerin. The third, which fails to retain its activity when so treated, causes tumors that are of relatively very slow growth (Chicken Tumor XVIII). Since in our experience the separation of a tumor-producing agent is largely a question of the growth's malignancy, it seems not improbable that with selective passage of the neoplasm an agent may eventually be obtained from it that is resistant to drying and glycerination.

The separation of etiological agents from three chicken tumors of such diverse character as those we have employed is strong evidence for the view that many other growths of the fowl have a like cause. It is hardly necessary to point out that were the latent period of Chicken Tumor XVIII, when produced by the specific agent, somewhat longer than the two to six months observed in the present investigation, or were the agent only very slightly more difficult to separate from the tissue by filtration, its presence would not have been demonstrated. Chicken Tumor XVIII would then have remained, with the sarcomata of the rat and mouse, among the transplantable tumors without a cause separable from tissue cells.

CONCLUSION.

A causative agent has been separated from three chicken tumors of very different sort, namely a spindle-celled sarcoma, an osteochondrosarcoma, and a spindle-celled sarcoma peculiarly fissured by blood sinuses. This was accomplished after the tumors had been transplanted repeatedly and their malignancy enhanced. Each of the tumor-producing agents is a distinct entity in that it gives rise only to growths of the precise kind from which it has been derived. All pass through Berkefeld cylinders impermeable at the same test to small bacteria, and two of the three retain their activity in tumor tissue that has been dried or glycerinated. All are strikingly dependent for their action on derangement of the tissue with which they are brought in contact. The general findings strongly

suggest that the agents are of about the same size, and of the same natural class. It is perhaps not too much to say that their recognition points to the existence of a new group of entities which cause in chickens neoplasms of diverse character.

EXPLANATION OF PLATES.[13]

PLATE 7.

FIG. 1. Tumors caused by a Berkefeld filtrate of an extract of Chicken Tumor XVIII (a spindle-celled sarcoma fissured by blood sinuses) in Ringer's solution (filtration experiment 2). The sternum has been cut away and the body of the fowl eviscerated. In the left pectoral muscles is the large, pale, primary growth. On both sides at the junction of the sternal and vertebral ribs is the neoplastic rosary described in the text. That on the right has been cut through vertically. Its individual nodules have coalesced.

PLATE 8.

FIG. 2. A section of one of the growths produced by a filtrate of Chicken Tumor XVIII (filtration experiment 2). The fissuring with blood channels, sometimes accompanied by intracanalicular growth, is characteristic of this tumor. The numerous black points in the channels are the nuclei of the red blood corpuscles.

FIG. 3. Large osteochondrosarcoma produced by the intramuscular injection of 4 c.c. of the Berkefeld filtrate of an extract of Chicken Tumor VII (filtration experiment 2). The fowl was killed when comatose eighty-seven days after the injection. Its emaciation should be noted.

PLATE 9.

FIG. 4. The growth shown in the preceding photograph, after it had been sawed open. Scattered amid the smooth, whitish cartilage is much bone with red marrow.

FIG. 5. An early stage of an osteochondrosarcoma produced by a filtrate of Chicken Tumor VII (filtration experiment 2). The fowl was killed when the tumor was first noted, eighteen days after injection. Here and there in the precartilaginous tissue, which has the general character of a spindle-celled sarcoma, the matrix of cartilage is in process of formation.

PLATE 10.

FIG. 6. Another portion of the growth illustrated in figure 5. The formation of cartilage is well advanced.

FIG. 7. A section of the growth shown in figures 3 and 4. The cartilage is in process of replacement by bone. Note the calcification and the abundant red bone marrow.

[13] All the microscopic sections were stained with methylene-blue and eosin.

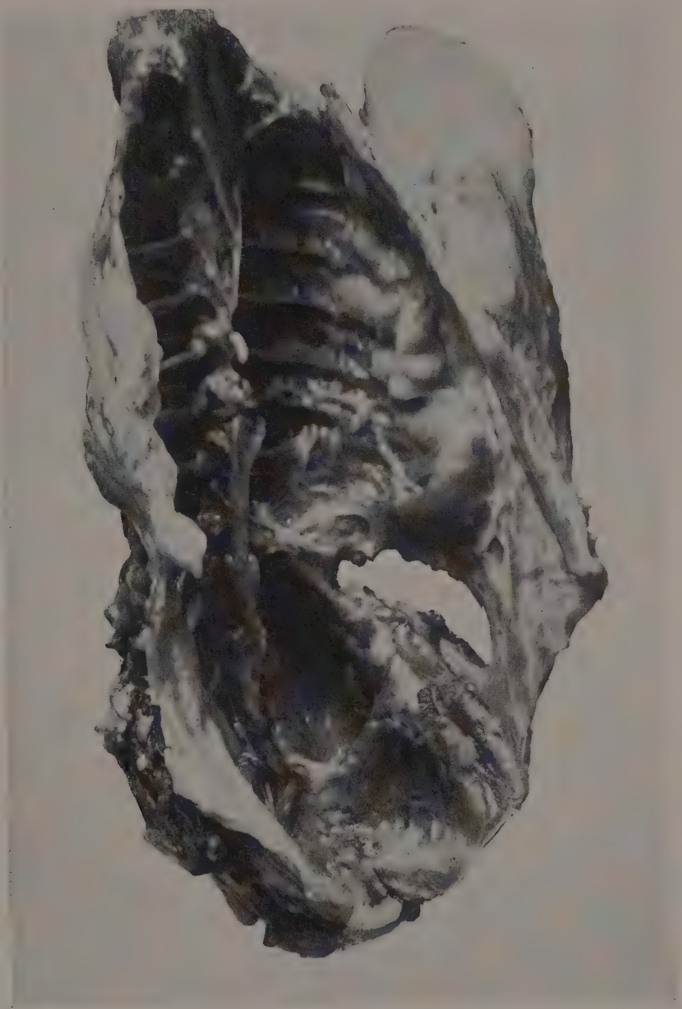


FIG. 1.

(Rous and Murphy: Causation of Chicken Tumors.)

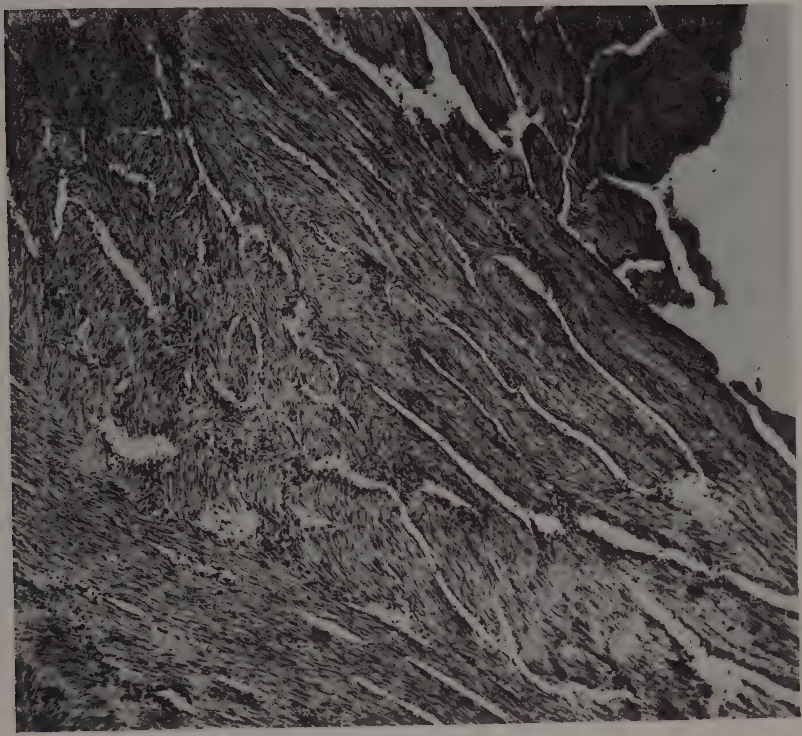


FIG. 2.

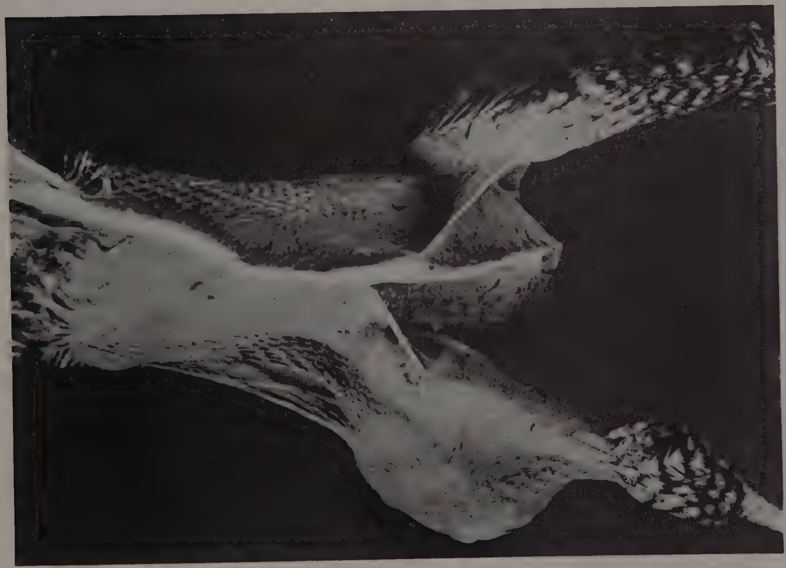


FIG. 3.

(Rous and Murphy: Causation of Chicken Tumors.)

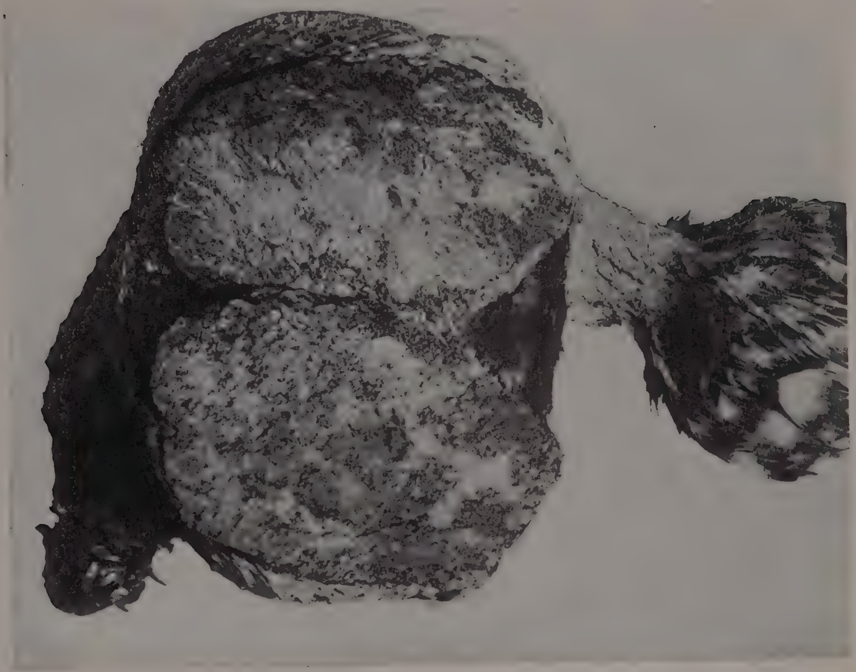


Fig. 4.

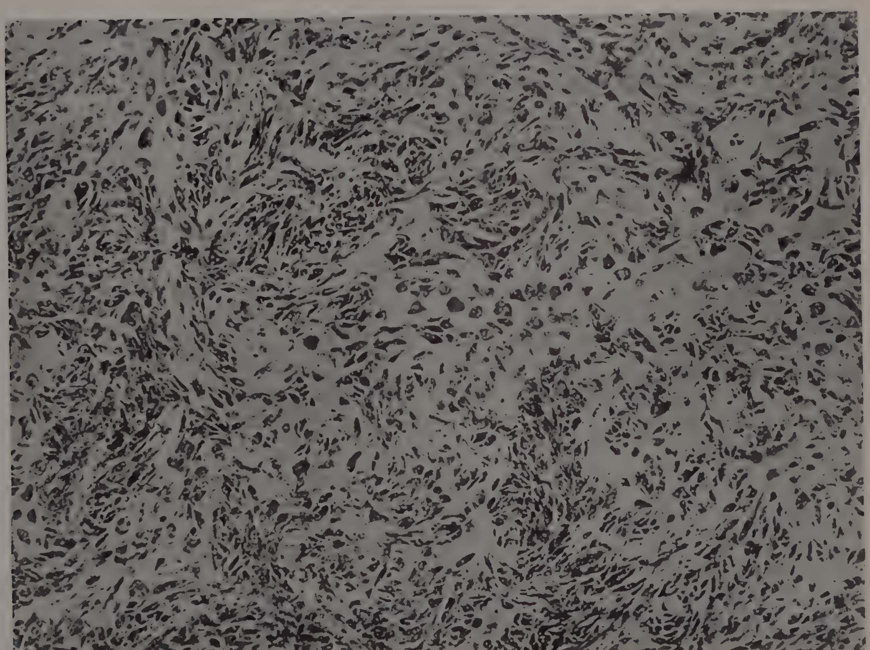


Fig. 5.

(Rous and Murphy: Causation of Chicken Tumors.)

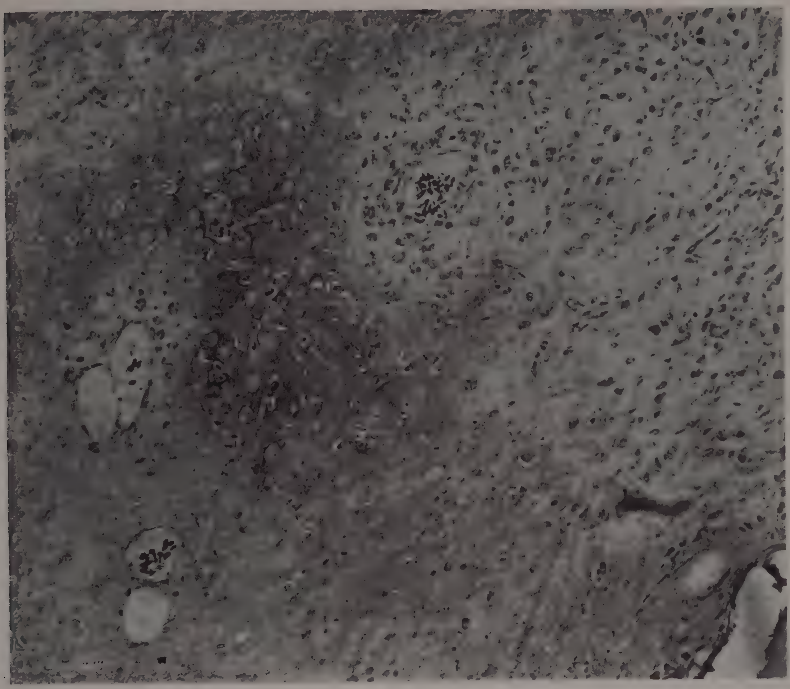


Fig. 6.

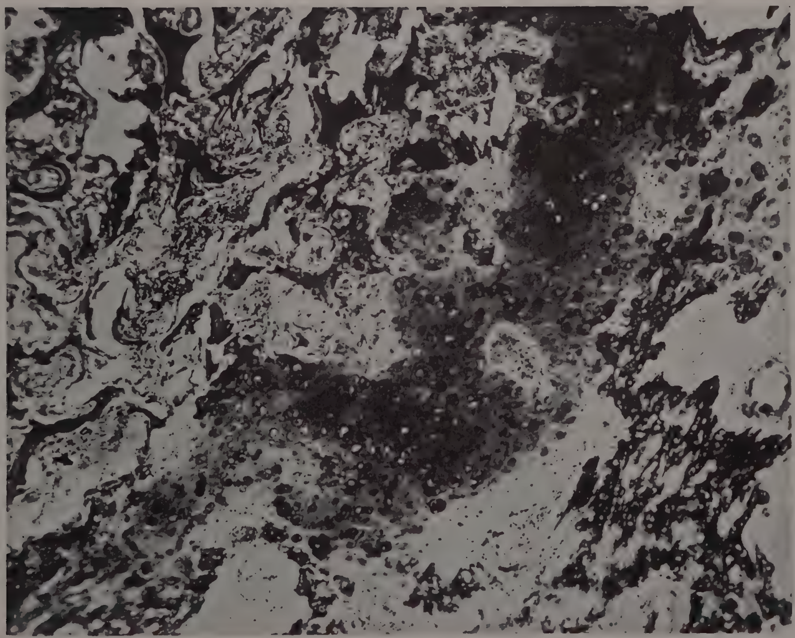


Fig. 7.

(Rous and Murphy: Causation of Chicken Tumors.)

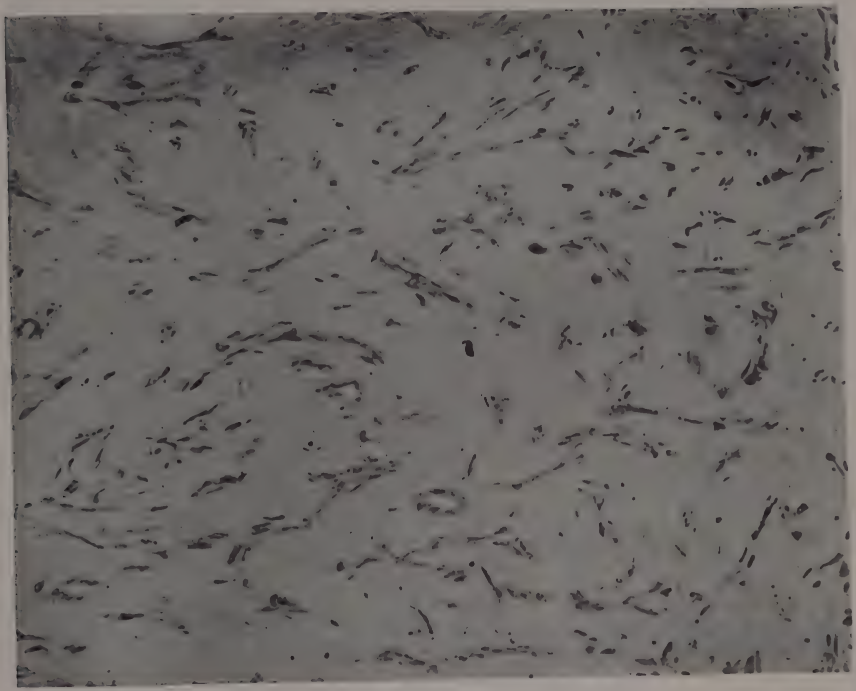


FIG. 8.

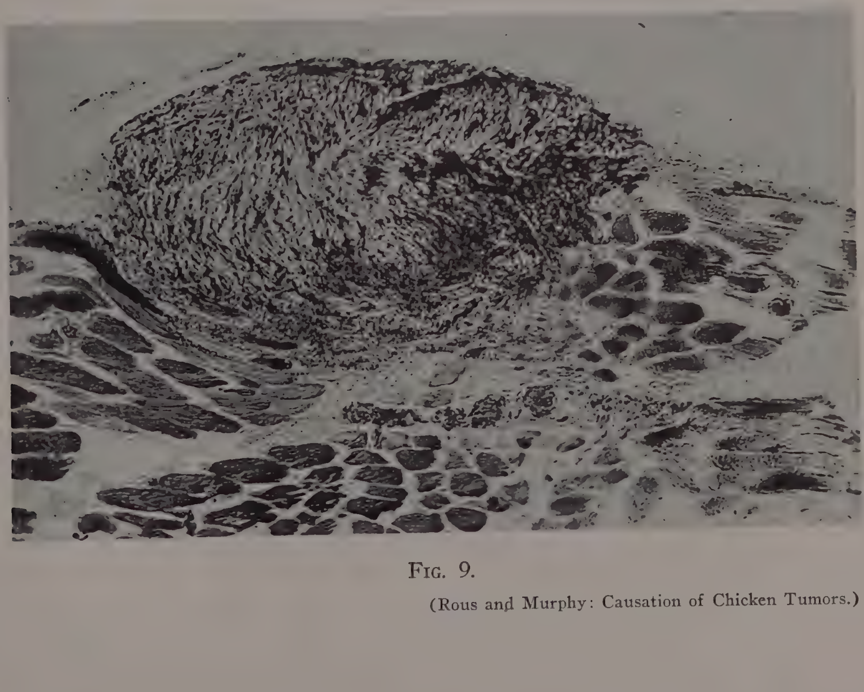


FIG. 9.

(Rous and Murphy: Causation of Chicken Tumors.)

FIG. 10.

(Rous and Murphy: Causation of Chicken Tumors.)

PLATE 11.

FIG. 8. Another portion of the same growth. An unusual intermediate stage in the formation of cartilage.

FIG. 9. A spindle-celled sarcoma developing after the injection of 0.5 c.c. of a filtrate of Chicken Tumor I. No *Kieselguhr* had been added to the filtrate. The growth arose in the sheath of the outermost pectoral muscle, where the needle had been thrust through, and it was excised eleven days after the injection, at which time it measured only 0.15 cm. in diameter. There was no recurrence. The tumor is distinctly sarcomatous, sharply localized, and is enlarging to a considerable extent by expansive growth, as shown by the way in which the muscle fibers are pressed to one side. Here and there it has begun to infiltrate. About it there is a slight round-celled reaction.

PLATE 12.

FIG. 10. The border of an osteochondrosarcoma that developed after the injection of a filtrate, on the basis of a *Kieselguhr* reaction. The fowl was killed while the tumor was yet very small and before cartilage had been laid down in it. The reactive tissue has been compressed into strata by the expansive growth of the tumor. The tumor cells, which are of fibroblastic character and occupy the upper half of the picture, are invading and replacing the giant-celled, reactive tissue about the *Kieselguhr*. Numerous fragments of this latter can be seen. With the destruction of the giant cells the fragments are set free within the tumor. Here they induce no evident reaction.

ON CERTAIN SPONTANEOUS CHICKEN TUMORS AS MANIFESTATIONS OF A SINGLE DISEASE.

I. Spindle-Celled Sarcomata Rifted with Blood Sinuses.*

By PEYTON ROUS, M.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

Plates 69 to 71.

Recently three transplantable chicken tumors distinct in character have been found to have a filterable cause.[1] The differences between these tumors are traceable to differences in the causative agents. Each agent gives rise in normal fowls to tumors of the sort from which it was isolated by filtration, and to tumors of this sort only. For example, the agent derived from a transplantable osteochondrosarcoma gives rise to sarcomatous tumors in which cartilage and bone are laid down. Certain minor variations, it is true, do occur in each tumor strain as intercurrent phenomena. The cells of the sarcoma known in our laboratory as Chicken Tumor 1 are, in some chickens, of very attenuated spindle form, again oat-shaped or almost round, again interspersed with sarcomatous giant cells; and the course of the disease varies somewhat in individual fowls. But the growth is always a spindle-celled sarcoma, and its modifications are not greater than those observed in certain rat and mouse tumors propagated only by transplantation and dependent on the survival of a single race of cells. Attempts to bring about variations by injuring the filterable agent have been unsuccessful, as have attempts to make it affect epithelium.

There is good ground to suppose that other tumors of the fowl besides those already studied are caused by filterable agents. The range in structure and behavior among chicken tumors is very wide.

* Received for publication, April 20, 1914.

[1] Rous, P., *Jour. Am. Med. Assn.*, 1911, lvi, 198. Rous, P., and Murphy, Jas. B., *Jour. Exper. Med.*, 1914, xix, 52.

Even when composed of cells of similar origin they often exhibit, like mammalian growths, a strikingly various structure and course. Must one suppose a distinct causative agent not only for each type of neoplasm as determined morphologically, but for the almost infinite number of variations in structure and behavior of such types? The present article and the one following it deal with this point. Briefly, it has been found that two spontaneous chicken tumors recently transplanted have each given rise to neoplasms identical in composite behavior with a tumor strain already under propagation. As will be shown in the present paper, the spontaneous tumor known as Chicken Tumor 38 of our series, seems to be a manifestation of a disease-complex already reported upon and known as Chicken Tumor 18.[2] This latter growth is a spindle-celled sarcoma, rifted in a characteristic manner with blood sinuses and tending to metastasize to the muscles, especially in the neighborhood of joints.

THE SPONTANEOUS TUMORS.

The spontaneous tumor No. 38 resembled the spontaneous tumor No. 18 only in the fact that it was a growth composed of spindle cells of connective tissue origin. The fowl carrying it was a well grown but emaciated Plymouth Rock hen. It was brought to the laboratory while yet alive. The irregular tumor mass, situated in the subcutaneous tissue between the left leg and the body, measured 10 by 6 by 5 centimeters, was imperfectly encapsulated, attached to the sheath of the thigh muscles, and just beginning to involve the skin. Strands extended between the leg muscles into the drumstick. At the center of the mass was a cavity with ragged walls, containing about forty cubic centimeters of clear, straw-colored fluid. The tumor tissue was finely striated, pinkish white, rather soft, and varied with many irregular, translucent areas of colliquation. There were no metastases. Histologically the growth was composed of strands of attenuated spindle cells with much collagen, sometimes in the form of ribbons (figure 1). A few round cells were scattered here and there in the growth. There was very little resemblance to the spontaneous tumor No. 18. This latter occurred

[2] Rous, P., and Lange, L. B., *Jour. Exper. Med.*, 1913, xviii, 651.

in the gizzard of a brown Leghorn fowl and metastasized to several points in the skeletal muscles. Both the primary and secondary tumors consisted of a very regular spindle-celled tissue, rifted to an extraordinary degree with blood sinuses into which the growth showed a tendency to extend, with result in an intracanalicular arrangement.

The fowl carrying Chicken Tumor 38 was killed and bits of the neoplastic tissue were implanted in the breast muscle of two normal Plymouth Rock fowls, in both of which a growth slowly developed. With repeated passage the tumor's rate of growth has increased somewhat, but like No. 18 it is still much less malignant than the simple spindle-celled sarcomata, Nos. 1 and 43. It is now growing in its fourth successive series of hosts. A filterable agent causing it, distinct from the tissue cells, has been demonstrated by three methods; namely, by drying, by glycerination, and by filtration through Berkefeld cylinders impermeable to small bacteria. The findings compared with those in the case of No. 18 are, briefly, as follows:—

COMPARISON OF THE TUMOR STRAINS.

Both growths, whether obtained by transplantation or by the action of the filterable agent as such, are, in the gross, solid, pinkish white, unencapsulated, firm, and markedly resistant to the knife. When growing in voluntary muscle they tend to bind the fibers and limit motion, a feature not observed in the case of the simple spindle-celled sarcomata already mentioned. Both growths are composed of attenuated spindle cells arranged, often very regularly, in bundles or strands with much collagen which is usually in the form of bands or ribbons (figure 2). Giant cells are not present. The rifting with blood sinuses, which was so important a feature of the earlier generations of Chicken Tumor 18, is now only occasionally seen in this growth. Absent at first from Chicken Tumor 38, it has recently been met with in several cases (figure 3). Histologically the two growths are at present practically indistinguishable.

Chicken Tumor 18 in its earlier generations showed a notable tendency to metastasize to the skeletal muscles, especially in the neighborhood of joints. The lungs, heart, and liver were affected

sometimes, though rarely. Among nine fowls which have thus far died of Tumor 38, one had secondary tumors in lung, liver, and gizzard. In three cases there were metastases in the skeletal muscles. The simple, spindle-celled sarcomata, Nos. 1 and 43, have never shown this feature. Of the three instances referred to, one had a nodule in the wing, a second both wing and leg metastases, while in the third the involvement was widespread. A small nodule was present in the lung, larger secondary growths in the muscles of the neck, legs, and hip, and a series of coalescing masses connected the junctions of the sternal and vertebral ribs, forming what may be called a neoplastic rosary (figure 5). The whole condition closely resembled that in a fowl which died of tumors produced by the injection of a filtrate of Chicken Tumor 18.[3]

Tumor 18, though spontaneous in a brown Leghorn fowl, grows much better in the Plymouth Rock variety, a fact only recently determined and still to be reported upon in full. Chicken Tumor 38 likewise succeeds better in Plymouth Rocks than in brown Leghorns. But the two cases are hardly to be compared, for Tumor 38 occurred spontaneously in a Plymouth Rock fowl, and, from what is known of the laws governing transplantation, might be expected to succeed best in hosts of this sort, as indeed it does. Tumor 18 acts against the rule, growing better in hosts of an alien variety.

THE FILTERABLE AGENTS.

A causative agent for Chicken Tumor 18, as distinct from the cells, has been demonstrated only by filtration experiments. The dried or glycerinated tumor tissue is incapable of causing the growth. Tissue of No. 38, dried or glycerinated, gives rise to the tumor in a considerable percentage of normal fowls injected, and this within a few weeks. The Berkefeld filtrate of an extract of the growth acts almost as quickly. Filtrates derived from No. 18, on the other hand, seldom cause a tumor until several months after the injection. The differences in resistance and activity of the causative agents, as thus indicated, are the only points of dissimilarity between the tumors at present.

[3] Rous and Lange, *loc. cit.*

CHICKEN TUMOR 27.

All in all, the findings give one good reason to suppose that the spontaneous chicken tumors, Nos. 18 and 38, are different manifestations of a single disease-complex. It has seemed possible that other expressions of this complex might be present among our forty-five spontaneous neoplasms of the fowl. A search shows that the growth known as No. 27 is probably such a case. The host, a brown Leghorn hen, had several large lumps in the muscles of the wings and legs which limited motion markedly, a small nodule in the gizzard, and a number of raised, sharply defined, plateau-like masses in the skin, some deeply pitted with feather follicles and one of them ulcerated. It was impossible to say which growth was primary. All consisted of a close textured, finely striated, firm, pink, sarcomatous tissue. At the time, the case appeared unique, and indeed among the spontaneous growths subsequently obtained none has given a similar picture. But among the many fowls dying of transplantation tumors of No. 18,—now in its eleventh successive series of hosts,—a single instance closely resembling that of No. 27 has been met with. The fowl, of the second transplantation generation, is mentioned in a previous article.[4] The discoid masses in the skin consisted, as in the case of Tumor 27, of a sharply defined, nearly homogeneous, spindle-celled, sarcomatous tissue in the looser layers of the corium, the masses in the muscle of the same sarcoma arranged for the most part in the familiar intracanalicular pattern (figure 4). Unfortunately no adequate attempt was made to transplant Tumor 27. The other spontaneous chicken tumors do not suggest, even remotely, the disease-complex of Nos. 18 and 38.

SUMMARY.

Two spontaneous chicken tumors, unlike in several important respects, have given rise on transplantation to neoplasms of identical character. The spontaneous growth, No. 18, situated in the gizzard, was a spindle-celled sarcoma rifted with blood sinuses into which it extended, with result in what may be described as an intra-

[4] Rous and Lange, *loc. cit.*

canalicular pattern. The metastases, which were in the voluntary muscles, showed the same peculiar structure. Tumor 38, occurring in the subcutaneous tissue of the groin, was a solid, spindle-celled sarcoma of rather close texture, with few blood vessels. Here and there were small areas of softening, and at its center was a large degeneration cyst with ragged walls, containing a clear fluid. There were no metastases. The transplantation tumors from both growths have been characterized by slow growth, tendency to metastasize to the skeletal muscles without involvement of the lungs, and a structure which at one time is that of a very regular spindle-celled sarcoma containing many bands and ribbons of collagen, and at another that of a sarcoma rifted with blood sinuses like the spontaneous tumor No. 18. At present the two strains are practically indistinguishable in appearance and general behavior. Both are caused by filterable agents. The agent causing No. 38, unlike that causing No. 18, retains its activity in tumor tissue which has been dried or glycerinated; and in a Berkefeld filtrate it is much the more active in causing tumors. These differences can hardly be thought of as constituting a fundamental distinction between agents which, to judge from their effects, are almost undoubtedly different strains of a single disease cause.

That chicken tumors of markedly different type have different filterable agents as their cause has been proved by experiments already reported. The present findings make it probable that, within certain limits, tumors of rather various character may be dependent upon a single agent. This assumption greatly simplifies the etiological problem. But the truth of the assumption for other instances than those described in the present article can only be determined by the study and comparison in many hosts of the disease-complexes of which each spontaneous chicken tumor is to be considered as an individual expression.

EXPLANATION OF PLATES.[5]

PLATE 69.

FIG. 1. Section of the spontaneous tumor No. 38. It is composed of spindle cells in strands, with abundant collagen. There is some round-celled infiltration.

FIG. 2. A solid growth of the third generation of transplants. The heavy black spots are artefacts.

PLATE 70.

FIG. 3. Portion of a growth that resulted from the injection into a normal fowl of tumor tissue that had been dried while frozen. The rifting with blood sinuses here shown has been found in several transplantation growths as well.

FIG. 4. Spontaneous Tumor 27. Section of one of the growths in the skeletal muscles.

PLATE 71.

FIG. 5. Secondary growths in a fowl of the first transplantation series of Tumor 38. The primary growths were situated in the pectoral muscles. They have been removed with the sternum except for a small portion of that on the left (*A*). There are metastases in both legs near the knee (*B*), in the neck muscles (*C*), and in the muscles within the bony trunk (*D*). A number of nodules coalescing into a thick cord (*E*) connect the junctions of the sternal and vertebral ribs.

[5] The microscopic sections were stained with methylene blue and eosin. The illustrations should be compared with those of Chicken Tumor 18 (Rous and Lange, *loc. cit.*).

FIG. 1.

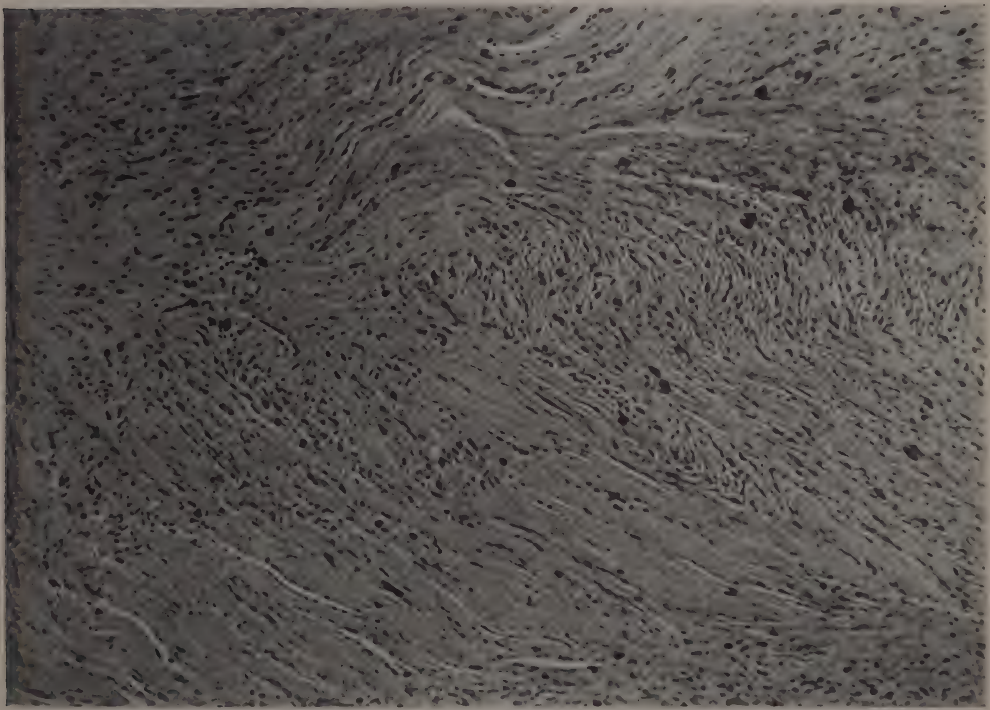


FIG. 2.

(Rous: Spontaneous Chicken Tumors.)

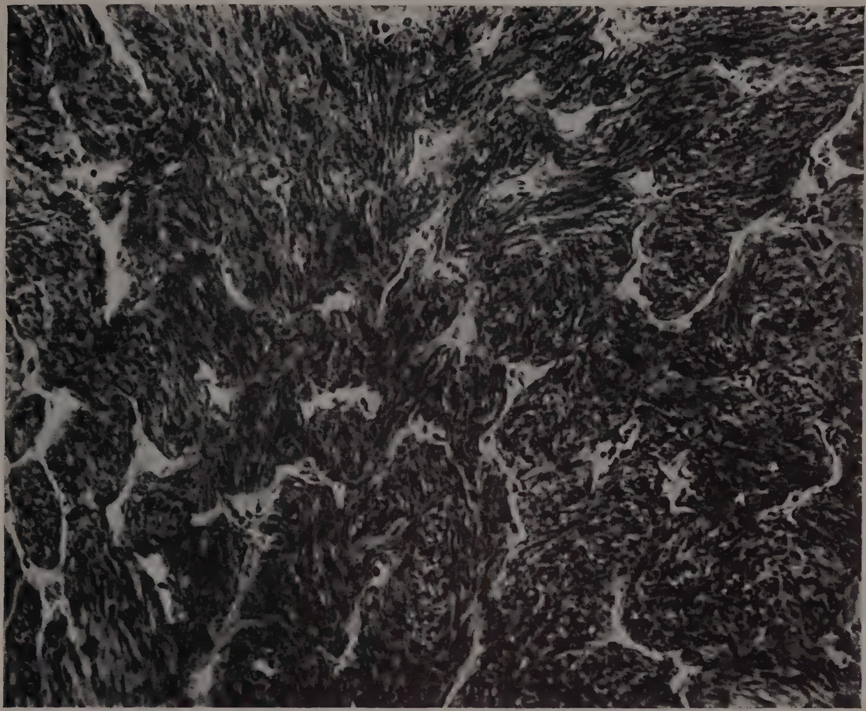


FIG. 3.

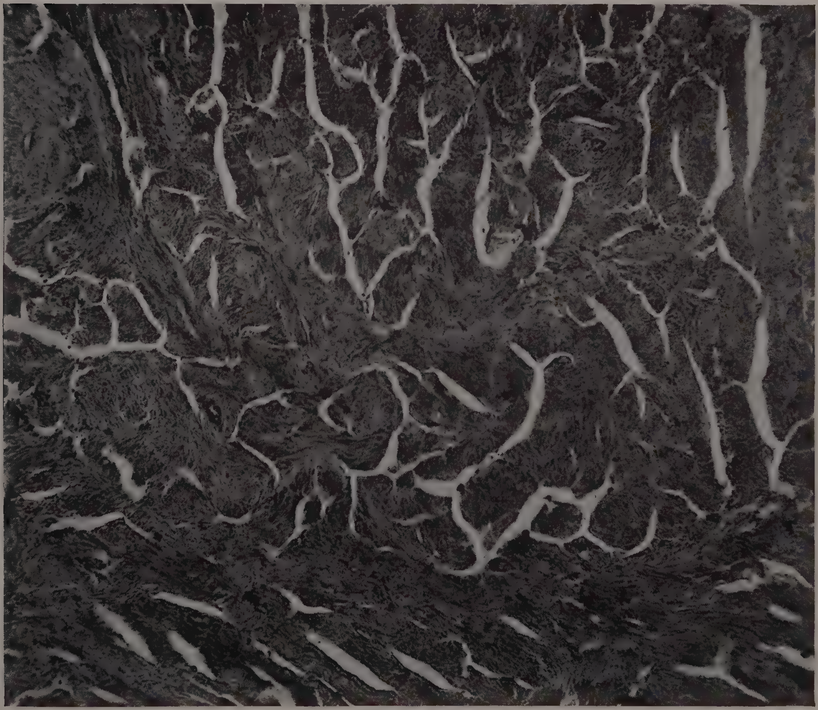


FIG. 4.

(Rous: Spontaneous Chicken Tumors.)

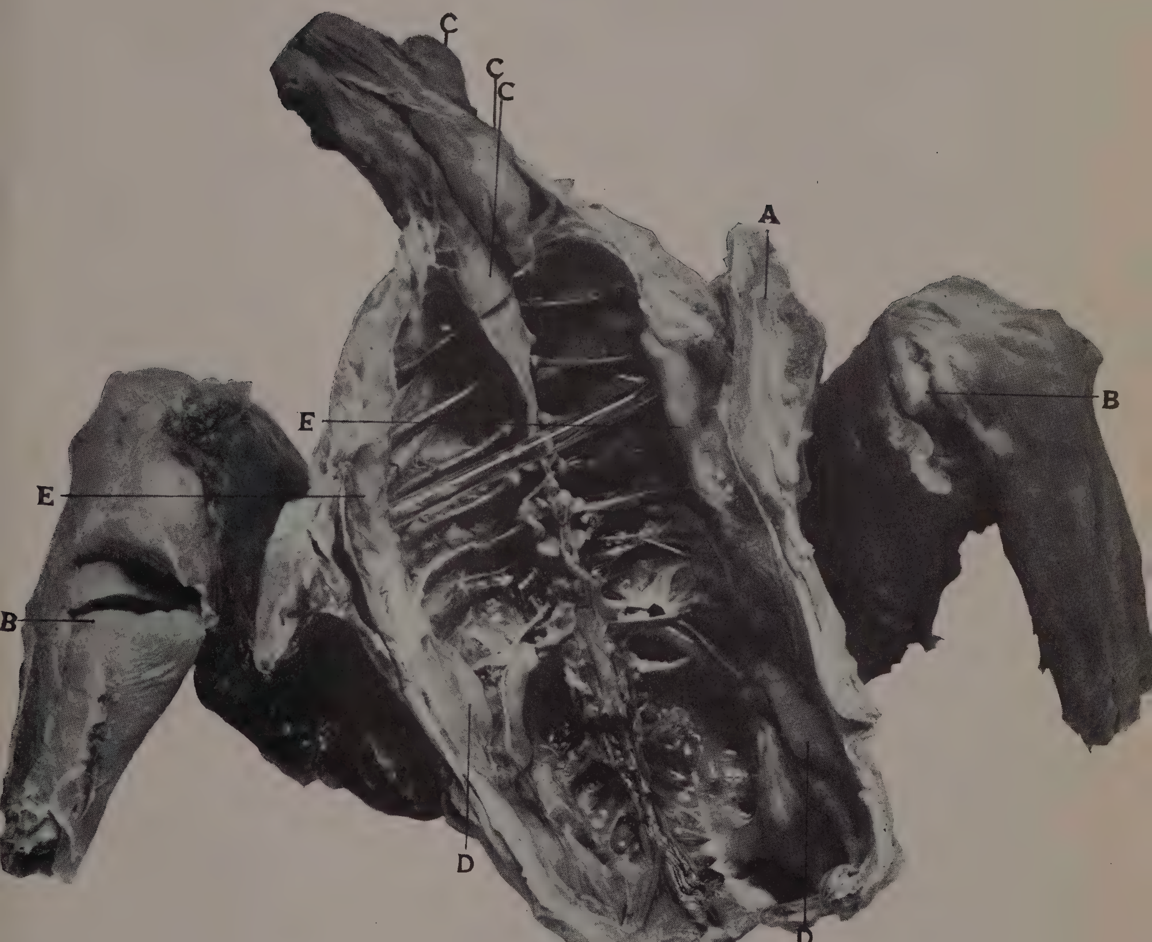


FIG. 5.

(Rous: Spontaneous Chicken Tumors.)

[Reprinted from the JOURNAL OF EXPERIMENTAL MEDICINE, Vol. XIX, No. 6, 1914.]

ON CERTAIN SPONTANEOUS CHICKEN TUMORS AS MANIFESTATIONS OF A SINGLE DISEASE.

II. SIMPLE SPINDLE-CELLED SARCOMATA.*

BY LINDA B. LANGE, M.D.

(From the Laboratories of The Rockefeller Institute for Medical Research.)

PLATES 72 TO 74.

Among the spontaneous chicken tumors recently brought to this laboratory there have been two spindle-celled sarcomata that have yielded, on transplantation, neoplasms similar respectively to two strains already under propagation. The resemblance of the growths derived from Chicken Tumor 38 to those derived from Chicken Tumor 18 is taken up in the preceding article.[1] They are spindle-celled sarcomata of protean character, often rifted with blood sinuses in a characteristic manner and showing a tendency to metastasize to the voluntary muscles. The subject of the present paper is Chicken Tumor 43, a simple spindle-celled sarcoma, apparently identical with Chicken Tumor 1.

The spontaneous tumor No. 38 differed considerably from the spontaneous tumor No. 18, and only after the growths had been observed in many hosts was their close similarity realized. Tumor 43, on the other hand, in its original form strikingly suggested Tumor 1, and the transplantation growths are practically identical with those of the latter. Both are produced by a filterable agent.

GROSS CHARACTERISTICS.

The original Chicken Tumor 43 occurred in a Plymouth Rock hen as a large nodular mass in the substance of the pectoral muscle, loosely attached to the lower end of the sternal keel, but not in-

* Received for publication, April 20, 1914.

[1] Rous, P., *Jour. Exper. Med.*, 1914, xix, 570.

volving the skin. The mass was fairly well defined, but devoid of a capsule. On incision the tumor tissue was, for the greater part, smooth, greyish pink, and firm, traversed by a few large blood sinuses, and well nourished throughout.

Pieces of the fresh tissue were inoculated into the pectoral muscles of two Plymouth Rock fowls by means of small trocars, the method employed also in subsequent transfers. The tumor grew in one of the two fowls. The rate of growth was slow at first, but on transplantation the malignancy increased rapidly, and in the fourth tumor generation the number of takes reached 100 per cent. The rate of growth as measured by the longest diameter was three times as rapid in the eighth as in the first generation. As contrasted with the firm almost gristly tissue of the earlier growths the more malignant tumors of later generations have been translucent and friable, and wet with a mucinous fluid. Hemorrhage into the tumors is frequent. In resistant fowls, on the other hand, the growth is firm, dense, nodular, and may undergo liquefaction resulting in cysts containing a clear mucinous fluid. With the progressive enlargement of the tumors the host emaciates, becomes cyanotic, and finally dies in coma.

Metastases were found in the original fowl in the heart and lungs, but not again until the fourth transplantation generation when they were also situated in the heart and lungs. Metastases have since been fairly frequent. They usually occur in the heart, lungs, and liver, less often in the spleen and kidney (figures 1 and 2). Implantation tumors on the heart and liver from a tumor growing through the body wall have been observed once.

MICROSCOPIC FINDINGS.

The tissue of the original growth is composed for the most part of slender spindle cells of somewhat irregular size, with pale, oval, vesicular nuclei often containing elongated or double nucleoli. Mitotic figures are fairly frequent. Scattered through the tissue are a few giant cells (figure 3). In some areas the cells are plumper and irregularly oval. The tissue structure varies, being very compact in some places and loosely meshed in others.

This picture has been fairly constant. Among the transplantation growths giant cells have been infrequent and the tumors have presented the general characters of a simple, spindle-celled sarcoma. In hosts relatively resistant, as shown by the behavior of the tumor, accumulations of small round cells are found, especially at the edge of the neoplastic tissue. The vigorously growing tumors are composed of spindle cells fairly uniform in size, shape, and arrangement. At the edge of the tumors there is practically no cellular reaction. When very malignant the tumor invades the muscle, not only by growing between the muscle bundles and fibers, but by penetrating the sarcolemma and replacing the muscle substance directly (figure 4). The metastases are histologically identical with the primary tumors.

ETIOLOGY.

Bacteriological cultures from the tumor tissue on the ordinary media have remained sterile under aerobic and anaerobic conditions. A causative agent separable from the tumor cells has, however, been demonstrated. The clear fluid obtained by filtering a thin suspension in Ringer solution of the finely ground tumor through Berkefeld filters holding back a test bacterium at the same filtration is capable of giving rise to tumors in normal fowls. These filtration tumors are identical with those from which the tissue for the emulsion was obtained. Tissue ground, frozen, and dried *in vacuo* over sulphuric acid, made up to the original bulk with distilled water, and injected into normal chickens, likewise causes tumors. Finally, the opalescent fluid obtained by centrifugalizing a thin suspension of the ground tissue in Ringer's solution may be mixed with glycerin and kept at 5° C. for many days without losing its ability to cause tumors. Mixtures containing 80 per cent. glycerin tested after ten days, and those containing 50 per cent. glycerin tested after twenty-one days were still active. Tumors arose from two to three weeks after inoculation of the material. As with the other chicken tumors, no case of cage infection has ever occurred.

RESEMBLANCE TO CHICKEN TUMOR I.

The spontaneous Chicken Tumor 43 and its transplanted growths closely resemble those of the strain known as Chicken Tumor 1.[2] They are of similar gross and microscopic structure, run the same course, metastasize, in general, to the same organs and have the same general action on the host. In appearance and behavior they are, indeed, indistinguishable. An etiological agent distinct from the living cells is easily demonstrated for both tumors by filtration, desiccation, and glycerination. In the absence of definite experiments upon the point it cannot be affirmed that both tumors have the same cause, yet a closer parallelism between two strains of the same disease obtained by transfer from separate instances occurring in nature could hardly be looked for. In this connection it is of interest to note that Chicken Tumor 13 (figure 5), a growth arising in the connective tissue of the foot, has some resemblance in its histology to Nos. 1 and 43, though it may not with certainty be classed with them. The attempt to propagate No. 13 was unsuccessful and nothing can be said as to its etiology.

SUMMARY.

The forty-third spontaneous chicken tumor received at this laboratory strikingly resembles the first and has given rise on transplantation to an entirely similar series of neoplasms. Tumors of both strains are due to a filterable agent which remains active in the dried or glycerinated tissue.

EXPLANATION OF PLATES.[3]

PLATE 72.

FIG. 1. Characteristic tumor filling out the left breast of the fowl. The right breast shows the emaciated state of the fowl. Metastases can be seen in the heart, lungs, and liver, as indicated by the arrows. This tumor arose from glycerinated tissue.

FIG. 2. Metastasis in the heart from a tumor of the eighth transplantation generation. There is a complete absence of a cellular reaction about the growth.

PLATE 73.

FIG. 3. Section of the original growth showing subcutaneous spindle-celled neoplasm with scattered giant cells.

FIG. 4. Tumor of the sixth transplantation generation invading striated muscle. The muscle fibers are directly replaced by tumor cells.

PLATE 74.

FIG. 5. Section of Chicken Tumor 13.

[2] Rous, P., *Jour. Exper. Med.*, 1910, xii, 696; 1911, xiii, 397.

[3] The microscopic sections were stained with methylene blue and eosin.

FIG. 1.

(Lange: Spontaneous Chicken Tumors.)

Fig. 2.

Fig. 3.

(Lange: Spontaneous Chicken Tumors.)

FIG. 4.

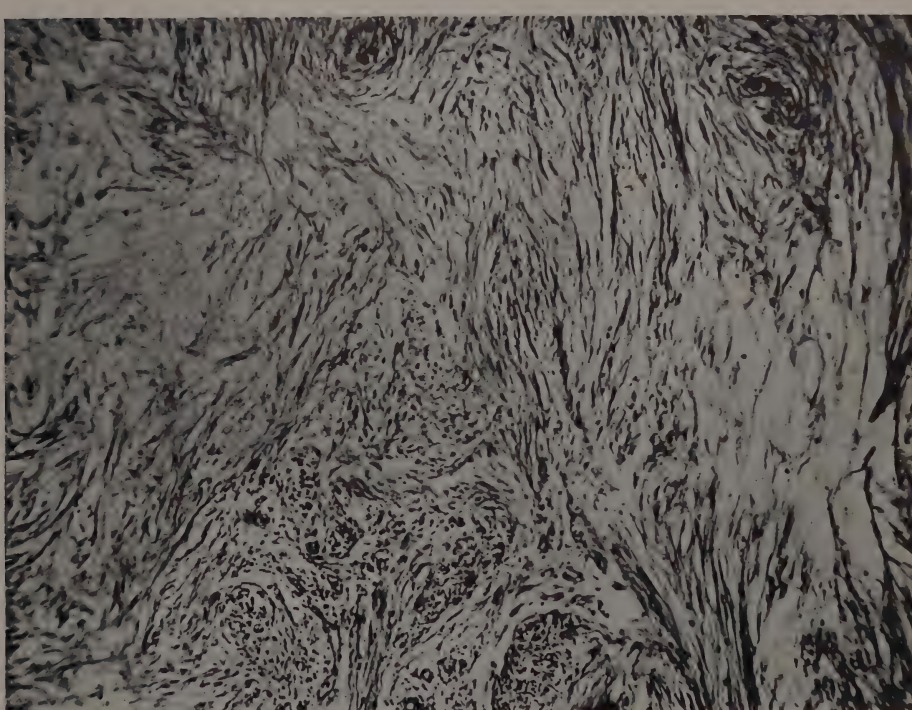


FIG. 5.

(Lange: Spontaneous Chicken Tumors.)

[Reprinted from the JOURNAL OF EXPERIMENTAL MEDICINE, Vol. XX, No. 4, 1914.]

ON IMMUNITY TO TRANSPLANTABLE CHICKEN TUMORS.*

BY PEYTON ROUS, M.D., AND JAMES B. MURPHY, M.D.

(From the Laboratories of The Rockefeller Institute for Medical Research.)

The observation that the transplanted tumors of mice sometimes retrogress has been provocative of much research; for in it the phenomenon of acquired resistance to neoplasms was first clearly recognized. Now we know that this resistance is not peculiar to tumors but is elicited by non-neoplastic tissues as well. A resistance attributable to a causative element in mammalian new growths has still to be demonstrated, as, indeed, has such an element. Causative agents for transplantable chicken tumors, on the other hand, have been found. Those thus far studied are filterable. In the light of this fact a comparison of the phenomena of resistance to chicken tumors and resistance to mammalian growths becomes of much interest; since it may well be that there exist gross differences that would prove the two of different etiology. The present paper is concerned with such a comparison. In addition there will be taken up the question of the relationship between the agents causing different chicken tumors as indicated by the specificity of the resistance to them.

We have used for the work three distinct chicken tumors, namely, a simple, spindle-celled sarcoma (Chicken Tumor I), an osteochondrosarcoma (Chicken Tumor VII), and a spindle-celled sarcoma curiously fissured with blood sinuses and showing a tendency to metastasize to the skeletal muscles (Chicken Tumor XVIII). Most of the data have been obtained with Chicken Tumor I. which has been longest in our hands.

NATURAL RESISTANCE.

Natural resistance to the avian tumors will be briefly dealt with, since it has already been reported upon in describing the growths.

* Received for publication, August 1, 1914.

Rat and mouse tumors, like the non-neoplastic tissues, can be successfully transferred under ordinary circumstances only to animals of the same species. This is true of chicken tumors as well. They will not grow in rats, mice, rabbits, or pigeons; and the spindle-celled sarcoma, the only one thus tested, will not grow in ducks. In fowls that are sick or emaciated the tumors do badly, either failing to develop after the inoculation, growing slowly, or retrogressing early. The same peculiarity has excited much attention in the case of mammalian growths. These latter grow best in young animals, and especially well in the new-born.[1] The influence of the age of the host upon chicken tumors has been tested only with the simple spindle-celled sarcoma. Young fowls have been found most susceptible as hosts for it, and in chick embryos it grows with extraordinary rapidity.[2]

Not a few mouse tumors are transplantable solely to animals of the variety in which the growth was spontaneous. A still greater specificity has been shown by Chicken Tumor I, which was transplantable at first only to blood relations of the original host and not to other varieties than the original until after months of propagation. The osteochondrosarcoma exhibits no preference for a special variety of fowl. The sarcoma rifted with blood sinuses shows what may be termed a reversed specificity, growing better in fowls of an alien sort (barred Plymouth Rock) than in the original brown Leghorn variety. This finding has been made the subject of a special paper.[3]

There exists an individual resistance to mammalian growths independent of all the factors thus far mentioned. Animals possessing it in its complete form fail to develop a tumor even though inoculated again and again. This is true of chicken tumors as well. But it is noteworthy in both cases that as the malignancy of the growth increases, owing to its sojourn in susceptible hosts, the number of animals insusceptible to it lessens. An individual naturally resistant to one form of mammalian tumor is frequently very susceptible to another. There is abundant evidence that this is true of avian growths as well.

[1] Unpublished work from this laboratory.

[2] Rous, P., and Murphy, Jas. B., *Jour. Am. Med. Assn.*, 1911, lvi, 741.

[3] Rous, P., and Lange, L. B., *Jour. Exper. Med.*, 1914, xx, 413.

Experiment I.—Nine fowls were employed, four of them normal Plymouth Rocks, and the remainder brown Leghorns naturally resistant to the rifted sarcoma, as is shown by its failure to develop in them on a previous inoculation. All were now inoculated in the muscle of one leg with a bit of a slowly growing simple sarcoma (Chicken Tumor I), in the other with the rifted sarcoma. The inoculation of the simple sarcoma was unsuccessful and after seventeen days a second inoculation was made at the same site with more malignant material. The rifted sarcoma was then just beginning to appear. The final results are shown in text-figure I.

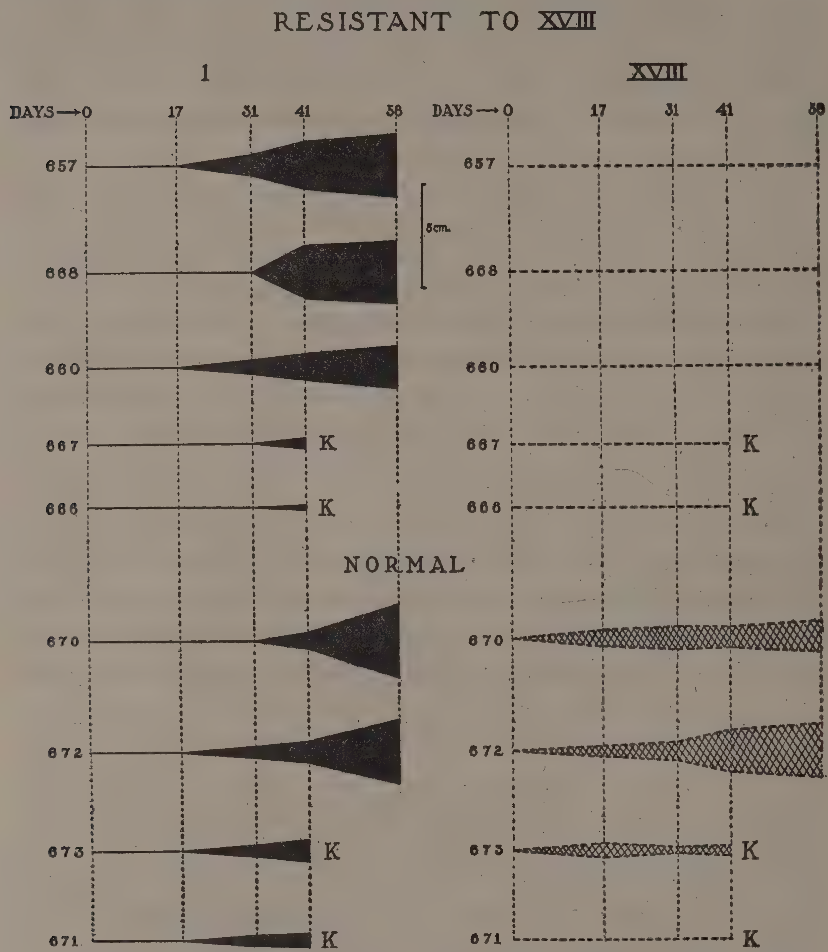
Text-figure I shows that fowls with a complete natural resistance to the rifted sarcoma and perhaps a slight acquired one (from the previous inoculation) were as susceptible to the simple sarcoma as normal fowls in which the rifted sarcoma grew well.

From all of the foregoing it is plain that the phenomena of natural resistance to chicken tumors are, in general, strikingly similar to those associated with rat and mouse tumors. The only apparent exception is in the tendency shown by the rifted sarcoma to grow better in fowls of an alien variety. Even here an instance somewhat similar may be found in mammals. Tyzzer bred together mice of two varieties, the one susceptible, the other insusceptible to a transplantable tumor of the Japanese waltzing mouse and found that the offspring of the F_1 generation were more susceptible than the susceptible parent. In the case of the rifted sarcoma the varieties of host tested were both the result of interbreeding several strains of fowls.

ACQUIRED RESISTANCE.

Some kinds of transplantable mammalian tumors grow progressively until the death of the animal; others after brief growth tend to become stationary and retrogress. The osteochondrosarcoma's behavior is of this latter sort. After a period of rapid enlargement as a chondrosarcoma in which spicules of bone gradually appear, it in most cases ceases to grow and is slowly absorbed. It not infrequently retrogresses after reaching a diameter of six or seven centimeters, but may take months to disappear, especially when it contains much bone. Only by the careful selection of tumors still growing has it been propagated. The simple spindle-celled sarcoma as a rule develops rapidly and progressively; but by the transplantation of slowly growing examples a retrogressing form may be obtained.

The rifted sarcoma develops slowly and with a considerable proportion of retrogressions.



TEXT-FIG. 1. Experiment 1. This shows that the simple sarcoma (Chicken Tumor I) implanted in fowls resistant to the rifted sarcoma (Chicken Tumor XVIII) grew as well as in normal fowls susceptible to the latter.

The time of appearance and rate of development of each tumor are shown by diagrams of which the width represents the diameter of the growth and the length its period of existence. A straight line indicates that no tumor developed. Cross-hatching indicates a rifted sarcoma, and solid black a simple sarcoma. The two are grouped in separate columns. The fowls are Nos. 657, 668, etc. K = killed.

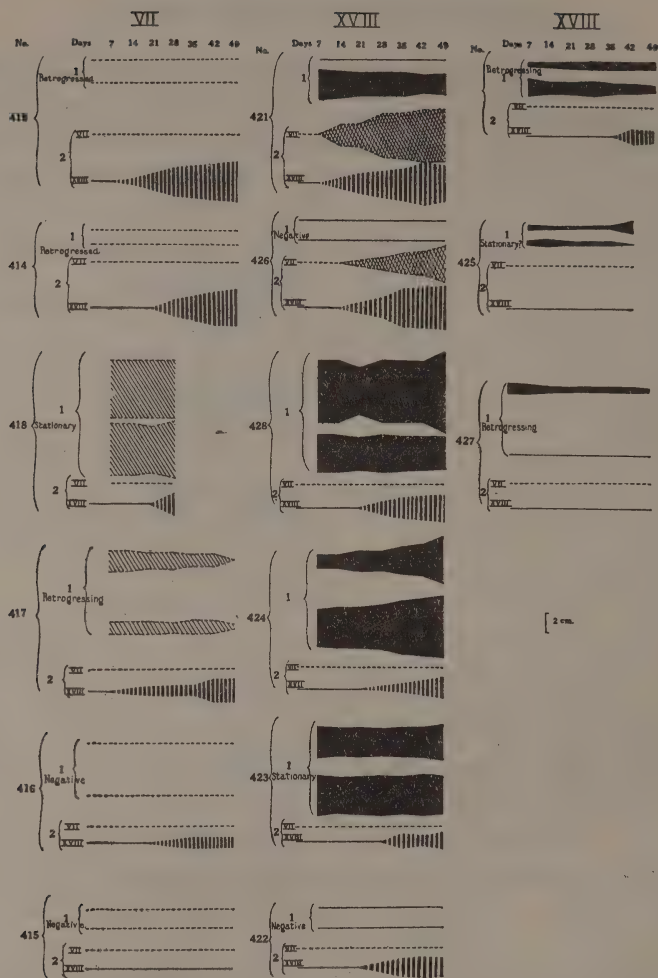
The amount of tumor material implanted has a marked influence on the course of all three chicken tumors. Retrogressing growths follow much more frequently the inoculation of single, small tumor bits than they do the inoculation of one to two cubic centimeters of the same tumor tissue, ground to a pulp. That dosage has an influence on the development and course of mammalian growths has long been known (Loeb, Clowes, and Baeslack).

Rats and mice in which tumors retrogress acquire resistance, as is shown by the fact that reinoculations within a few weeks usually fail of success. Some mouse tumors confer resistance on the host while they are still growing. This is especially true of tumors of retrogressing tendency. In the case of certain other neoplasms the factors which determine the course of the disease are so balanced that by mechanical means a stationary tumor may be made a growing one;[4] or reinoculations into a host in which the growth is stationary or retrogressing may be successful.

Exactly the same phenomena have been noted of the chicken tumors. The spindle-celled sarcoma grows rapidly and the success of secondary inoculations shows that it produces no notable concomitant resistance. The few individuals in which it is absorbed are usually resistant for a considerable time. The osteochondrosarcoma, a growth which tends to retrogress, produces a strong concomitant resistance (text-figures 2 and 5). Chickens in which it has been present for several weeks are always absolutely resistant on secondary implantation, and this at a period when the primary tumor is still growing. The slowly developing, rifted sarcoma often becomes stationary for long periods and then starts to grow again. By the use of malignant material hosts in which this growth is stationary or even slowly disappearing may sometimes be successfully reinoculated.

The resistance induced by the retrogression of a rat or mouse tumor is in part a pan-resistance but is most effectual against tumors of the same sort. Whether a pan-resistance to chicken tumors follows their retrogression has not been determined, but certainly much of the resistance is specific, as the following experiment shows.

[4] Loeb, L., *Jour. Med. Research,* 1901, vi, 28.



Text-Fig. 2. Experiment 2. This text-figure illustrates the fact that acquired resistance to the rifted sarcoma (Chicken Tumor XVIII) is slight as compared with that to the osteochondrosarcoma (Chicken Tumor VII); and it shows furthermore that resistance to the latter growth is to a large extent specific. All of the fowls (Nos. 419, 414, etc.) had been inoculated previously at two points. The character of this first inoculation (Chicken Tumor VII or Chicken Tumor XVIII) has determined the grouping in columns. For each fowl there are four diagrams representing tumor growth, or, in its absence, four lines. The diagrams bracketed together as 1 record the tumors of first inoculation; those bracketed as 2 record those of the second, the latter comprising an implantation with both Chicken Tumors VII and XVIII. The diagrams of the rifted sarcomata (Chicken Tumor XVIII) of first inoculation are given in solid black, those of the second in heavy hatching. A lightly hatched diagram indicates an osteochondrosarcoma of the first inoculation, and a cross-hatched one a tumor of the same sort following the second inoculation.

Experiment 2.—Six fowls previously inoculated with the osteochondrosarcoma and nine inoculated with the rifted sarcoma were chosen for this experiment. Some carried growths that were enlarging, some retrogressing growths, and others had shown themselves naturally resistant. All were now inoculated with the rifted sarcoma in the wing muscles of one side and with the osteochondrosarcoma at the same spot on the other side. 0.1 c.c. of a suspension of the fresh tumor tissue in Ringer's solution was used in each case. The course of the old tumors and the development of the new are shown in text-figure 2.

It will be seen from text-figure 2 that all of the fowls previously inoculated with the osteochondrosarcoma were now resistant to it. The malignancy of the material employed is proved by the rapidity with which it gave rise to tumors in two fowls previously implanted with the rifted sarcoma. This latter tumor grew in all but one of the fowls resistant to the osteochondrosarcoma. It also grew in seven of the nine hosts previously inoculated with a growth of its own sort. In one fowl the tumor of the first inoculation was actually retrogressing while that of the second enlarged.

When implanted simultaneously in the same host the chicken tumors preserve their character unchanged. The simple sarcoma metastasizes, as usual, to the lungs and other viscera, and the rifted sarcoma still gives secondary growths in the muscles, the source of each dissemination being clearly traceable from its histology. Sometimes one tumor grows rapidly whereas the others do badly or fail to grow (text-figure 4). So too it is with neoplasms of the rat and mouse. In a previous article the fact has been pointed out that the histological signs of resistance to these latter are identical with those to chicken tumors when allowance is made for the peculiarities of the two classes of host.[5]

Despite the efforts of many workers an immune principle effective against rat and mouse tumors has yet to be demonstrated in the blood of animals recovered from these growths. Crile and Beebe[6] succeeded in curing dogs of infectious lymphosarcoma by transfusing to them blood from other dogs in which the growth had retrogressed; but the lymphosarcoma has characters which distinguish it from the true neoplasms. Nevertheless, attempts to cure chicken tumors by means of transfusion have seemed advisable. Five fowls

[5] Rous, P., and Murphy, Jas. B., *Jour. Exper. Med.*, 1912, xv, 270.
[6] Crile, G. W., and Beebe, S. P., *Jour. Med. Research*, 1908, xviii, 385.

in which a relatively non-malignant form of the simple sarcoma was developing as the result of inoculation were bled from thirty-five to sixty-five cubic centimeters and an equal or slightly larger amount of blood was transfused to them from resistant fowls. In these latter the simple sarcoma had retrogressed and several intraperitoneal inoculations of sarcomatous tissue had from time to time been made without yielding tumors, a fact confirmed at autopsy. Transfusion was done at a time when resistance to the sarcoma may be supposed to have been at its greatest, that is to say, some two to three weeks after a massive injection of sarcomatous tissue. But in the fowls receiving the blood the tumors grew quite as well as in untransfused controls.

It is well known that not only does the retrogression of a mammalian tumor render the host unfavorable for subsequent tumor grafts but that injections of normal tissues, of normal blood even, will act to this end. Embryonal tissue is especially effective. In our experience the injection of hashed chick embryo does not confer resistance to the spindle-celled sarcoma of the fowl. But the tumor used was very malignant and may not have been sufficiently sensitive as an indicator.

Thus far the chicken tumors have been considered simply as transplantable new growths. The phenomena of acquired resistance to them resemble such as are seen under like conditions in the case of mammalian growths and suggest no more than these the presence of a causative agent distinct from the tumor cells.

RESISTANCE TO THE TUMOR-PRODUCING AGENTS.

By a special method there have been demonstrated two distinct forms of resistance against the simple sarcoma when it is transferred by grafting,—the one directed against the transplanted tumor cells, the other against the growth's causative agent.[7] Resistance of the latter sort will come into consideration in the findings now to be discussed.

With the exception of Königsfeld[8] workers with mammalian tumors have found that neoplastic tissue killed by drying fails to in-

[7] Rous, P., *Jour. Exper. Med.*, 1913, xviii, 416.

[8] Königsfeld, H., *Centralbl. f. Bakteriol., 1te Abt., Orig.*, 1914, lxxiii, 316.

duce resistance against subsequent grafts. We have repeatedly attempted to induce with dried tissue resistance to the spindle-celled sarcoma of the fowl. The growth's causative agent remains active after drying, so it was necessary to make the first inoculations with material rid in some way of its tumor-producing property. The dried and powdered tissue was taken up in distilled water, heated at 60° C. for fifteen minutes, and injected intraperitoneally. For the later injections material submitted to 55°, 53°, or 50° C. for fifteen minutes, and finally unheated material, was used. Several groups of fowls were employed, but few came to the eventual test with the implanted growth, because nearly all developed tumors following the inoculation with unheated, dried tissue. Those remaining may well have been naturally resistant. If any protection is elicited by the injection of dried material in which the agent exists in attenuated form, it must certainly be very slight.

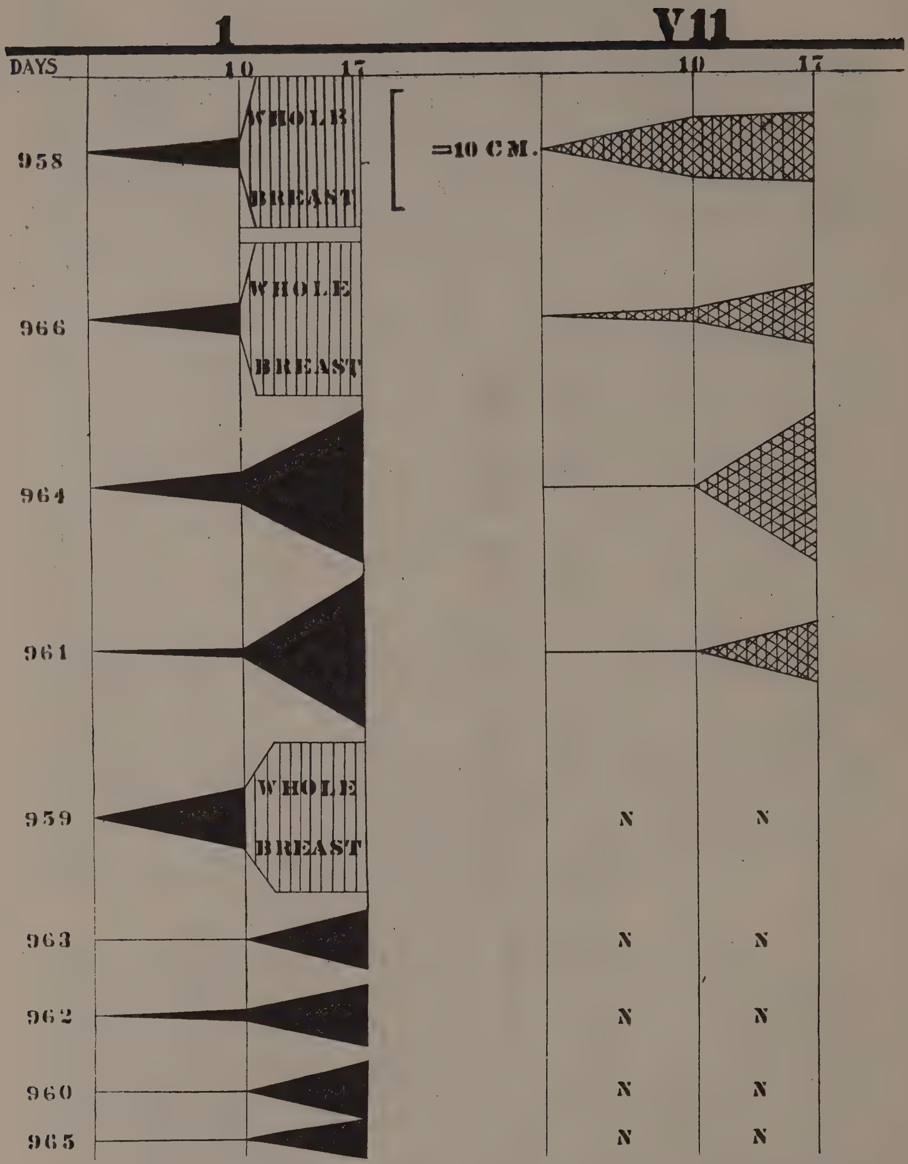
SPECIFICITY OF THE RESISTANCE.

That there exists a natural resistance to the agents is shown by their failure to produce tumors in some hosts. The question arises as to how far this resistance is specific.

Experiment 3.—Nine healthy Plymouth Rock fowls were inoculated, in one breast with 0.5 c.c. of a suspension of the dried tissue of an osteochondrosarcoma (Chicken Tumor VII), in the other breast with 0.1 c.c. of a like suspension of the dried spindle-celled sarcoma (Chicken Tumor I). The suspensions were made by rubbing up 1 gm. of dried tumor tissue in 9 c.c. of distilled water. The difference in dosage was to compensate for differences in the malignancy of the tumors. The results will be found in text-figure 3.

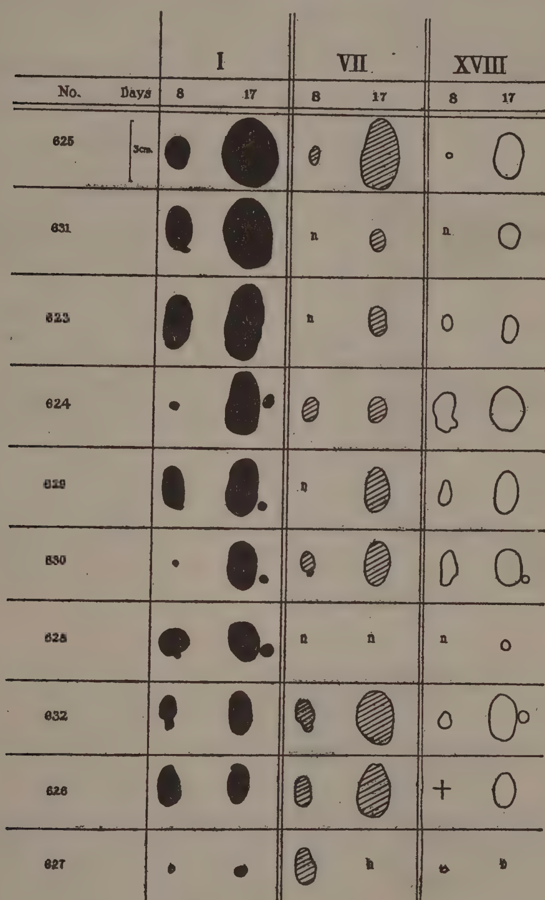
It is evident from text-figure 3 that the agents of the simple sarcoma and the osteochondrosarcoma are largely influenced by the same factors of natural resistance. In the experiment which it illustrates the period which elapsed before the appearance of a palpable tumor was so nearly the same for the two growths that the results can scarcely be referred to concomitant resistance induced by one tumor and effectual on the other.

With the rifted sarcoma a test of the above sort has not been possible because its agent is obtained apart from living cells only inconstantly and with difficulty. Comparative transplantation has been resorted to but this introduces a factor of error in that there



TEXT-FIG. 3. This shows that the same factors of natural resistance influence the activity of the agents causing two different chicken tumors. The fowls (Nos. 958, etc.) were inoculated in one breast with dried material of the simple sarcoma (Chicken Tumor I), in the other with that of the osteochondrosarcoma (Chicken Tumor VII). The diagrams are black for the simple sarcoma, cross-hatched for the osteochondrosarcoma.

are transferred with the agent tumor cells strange to the new hosts yet capable of active proliferation in many of them. With such a large disturbing element one would scarcely expect to learn much regarding the specificity of resistance to the agents. The following experiment gives evidence for the correctness of this view.

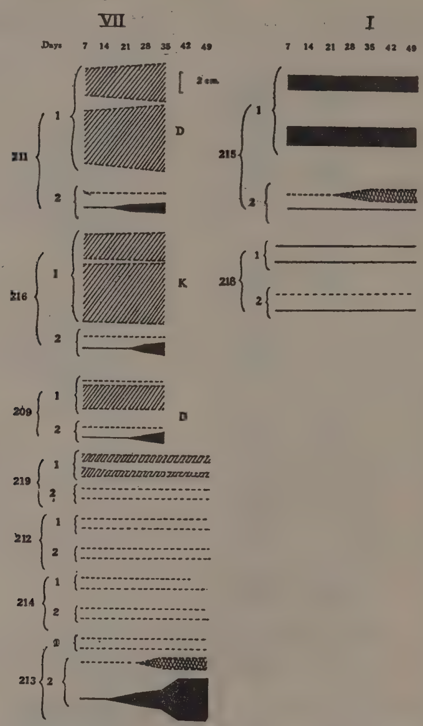


TEXT-FIG. 4. Fowls Nos. 625, etc., were inoculated simultaneously at different points with all three chicken tumors (I, VII, and XVIII). The text-figure shows that the growths varied independently of one another.

Experiment 4.—Ten healthy Plymouth Rock fowls were inoculated at different spots with all three chicken tumors (I, VII, and XVIII) in the amount of

0.1 c.c. of the finely ground fresh tissue. The sites chosen were in the muscle of both breasts, and the arrangement of the inoculations was varied from fowl to fowl. The results are shown in text-figure 4.

It will be seen from text-figure 4 that the tumors varied independently of one another. The findings as regards the simple sarcoma and the osteochondrosarcoma give no hint of the relationship seen in text-figure 3.



TEXT-FIG. 5. This text-figure has to do, like text-figure 2, with reinoculations; and the same general explanation holds good for it. The results of the first inoculation are given in the bracket 1 and those of the second in 2. The black and hatched diagrams are those of the simple sarcoma and the osteochondrosarcoma, respectively. The second inoculation was made with dried material of each growth. It will be seen that the agent of the simple sarcoma failed to give rise to tumors in fowls in which this growth had done badly on previous inoculation, whereas it caused growths in fowls resistant to the osteochondrosarcoma. The resistance against the latter tumor growth is also largely specific. Of seven fowls previously inoculated with it but one was susceptible on second inoculation. This fowl, No. 213, was supposed to be naturally resistant because of an unsuccessful inoculation some weeks previously, but the agents of both tumors engendered growths in it.

SPECIFICITY OF ACQUIRED RESISTANCE TO THE AGENTS.

Obviously the resistance acquired by a fowl in which a tumor has retrogressed must be effectual not only against the tumor cells but against the associated agent,—else this latter by acting on the cells of the host would produce a tumor. The following experiment indicates that acquired resistance to a tumor-producing agent is largely specific.

Experiment 5.—One fowl in which the simple sarcoma had retrogressed, one in which it was stationary, and four fowls carrying the osteochondrosarcoma were employed. They were inoculated, in one breast with 0.1 c.c. of a thin paste made by rubbing up dried tissue of the simple sarcoma with Ringer's solution, in the other with 0.5 c.c. of a similar paste of the dried osteochondrosarcoma.

As text-figure 5 shows, the four fowls carrying the osteochondrosarcoma evinced a complete resistance to it on secondary inoculation, whereas the simple sarcoma developed in three of them. The opposite result was obtained with the fowls in which the simple sarcoma had retrogressed or was stationary. Both now proved resistant to this growth, but in one the osteochondrosarcoma developed. The fact that the agent of the osteochondrosarcoma is relatively inactive renders the result more striking.

SUMMARY.

The phenomena of natural and acquired resistance to transplanted chicken tumors strikingly resemble those observed in the case of transplanted mammalian growths; and no more than those do they suggest that the tumors have an extrinsic cause.

That there may exist in fowls implanted with a chicken tumor a resistance directed against the tumor-causing agent distinct from the resistance manifested against the alien tumor cells has been shown in a previous article.[9] Both sorts of resistance are present in a fowl in which a tumor has retrogressed, the resistance in such an instance being acquired. That directed against the agent is largely specific, giving little if any protection against the agents

[9] Rous, P., *loc. cit.*

causing other tumors. There is some evidence that the conditions upon which a fowl's natural resistance depends are the same for the agents causing different chicken tumors.

It has proved impossible to protect chickens against the agent causing the simple sarcoma by injecting them with dried tumor material in which this agent has been attenuated by heat. The transfer of blood from resistant fowls to fowls with growing tumors is in our experience void of effect on the tumors.

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EXPERIMENTS ON THE PRODUCTION OF SPECIFIC ANTISERA FOR INFECTIONS OF UNKNOWN CAUSE.

II. THE PRODUCTION OF A SERUM EFFECTIVE AGAINST THE AGENT CAUSING A CHICKEN SARCOMA.

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The work detailed in Part I of this paper has served to demonstrate the theoretical usefulness of exhausted sera to combat infections, but it presents no instance of a serum actually resulting from the direct immunization of animals by injections of infected tissue. Such an instance is highly desirable. It has been furnished through experiments with a transplantable chicken sarcoma,[1] known in our laboratory as Chicken Tumor I, which has a filterable agent as its cause. The exact nature of the filterable agent is unknown, but its general characters would seem to place it with the microorganisms.[2] The tumor is a typical sarcoma, highly malignant, and as a rule rapidly fatal to fowls developing it after an implantation with neoplastic tissue or inoculation with the Berkefeld filtrate of a tumor suspension. Some individuals are primarily insusceptible, and in some the growth develops slowly, and eventually retrogresses. The latter fail ordinarily to develop a sarcoma when reinoculated. Repeated unsuccessful attempts have been made to demonstrate antibodies in the blood of fowls in which a growth has retrogressed, and to render others immune to the tumor by injections with heated or dried neoplastic tissue.[3] The tumor cannot be transmitted to geese, ducks, pigeons, or mammals; but attempts to develop an antiserum by the immunization of such animals have been blocked through failure to obtain the tumor-

[1] Rous, P., *J. Exp. Med.*, 1910, xii, 696; *J. Am. Med. Assn.*, 1911, lvi, 198.

[2] Rous, P., and Murphy, Jas. B., *J. Am. Med. Assn.*, 1912, lviii, 1938.

[3] Rous, P., and Murphy, Jas. B., *J. Exp. Med.*, 1914, xx, 419.

producing agent in culture. The employment in these alien species of the neoplastic tissue itself as an antigen, or a filtrate from such tissue, elicits, of course, anti-chicken elements in the immunized individual.[4] The method of specific absorption to obtain an antiserum here finds a direct application.

Immunization of Animals.—The blood of fowls carrying the chicken tumor often contains during the last few days of life the causative agent of the disease; and in the sarcomatous tissue the agent is regularly present in large quantity. Both blood and tissue could therefore be used in the immunization, which was desirable in order to insure the production of a strong anti-chicken serum. Chickens moribund with the growth were bled to death under aseptic conditions, the blood was citrated, and the tumor tissue itself was ground with sand and suspended in Locke's solution just prior to injection. As the causative agent of the growth will withstand repeated freezing and thawing and retains its activity for a long period at low temperature, the material often was kept in the frozen state for days or weeks prior to use.

The first attempts to obtain an antiserum were made with rabbits. A number of these animals were injected intravenously on 3 successive days with a tumor extract in salt solution, and thereafter intraperitoneally every 6 days with citrated chicken blood and a suspension of tumor tissue. But though the serum soon acquired a high content of chicken hemolysins and hemagglutinins it had not the least neutralizing effect on the tumor-causing agent present in Berkefeld filtrates of suspensions of the sarcoma tissue. For this reason work with rabbits was at length discontinued.

Implanted bits of the chicken sarcoma perish at once in mammals, whereas in ducks and pigeons they grow for some days before retrogressing and may form quite large nodules. It seemed from this fact not improbable that birds would prove more favorable than rabbits as producers of tumor antibodies, owing to what might be considered as a partial susceptibility on their part to the neoplastic disease. For Flexner and his associates[5] have shown that in the case of poliomyelitis an immune serum is obtained only in species susceptible to the infection. Geese were used, therefore, in the further attempts to obtain an antiserum. Their immunization was carried out as follows:

Goose A received three intravenous injections on successive days of mixed tumor suspension and citrated blood from fowls moribund of the growth, followed thereafter every 6 or 7 days by intraperitoneal injections of the same material. Goose B was given the same sort of material, but only into the peritoneal cavity. From time to time both birds were bled from a wing vein and the sera compared

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[4] Bailey encountered this difficulty in experiments on complement fixation with the serum of pigeons inoculated with the growth (Bailey, C. H., *Med. Rec.*, 1915, lxxxviii, 403).

[5] Personal communication from Dr. Flexner.

in neutralizing properties with those of three normal geese. During a long period of immunization no differences could be noted, but on the contrary a similarity in the five sera so entire as to indicate that the blood of one normal goose is just like that of any other in its effect, or rather lack of effect, on the tumor-producing agent. At length, as will be seen from the experiments now to be cited, an immune principle became demonstrable in the serum of the injected birds.

Experiment 1.—The immunized geese, A and B, were bled for serum 84 and 66 days respectively after immunization was begun, and 9 days after the last injection of tumor material. Goose A had received three intravenous and ten intraperitoneal injections, while Goose B had but nine injections, all intraperitoneal. Two normal geese (a and b) were bled at the same time and to an equal amount; namely, 75 cc. The sera were inactivated as usual, and all were submitted to absorption with similar portions of washed chicken red cells, as follows:

Mixture.	Hemagglutination.	
	Immune sera.	Normal sera.
25 cc. of goose serum + 5.2 cc. of chicken red blood cells incubated 1 hr. and	+	0
serum transferred to 4 cc. of chicken red blood cells, incubated 1 hr.	Tr.	0

Cultures taken after the second absorption proved sterile. Preliminary tests showed that the untreated immune serum failed to hemolyze chicken cells when chicken serum was used as complement, whereas these were rapidly destroyed in the presence of guinea pig complement. Consequently the latter was used in the titrations that follow.

Anti-Chicken Titer of the Sera. Hemolysis.—0.2 cc. of inactivated serum in graded dilutions + 0.2 cc. of 1 in 10 guinea pig complement + 0.2 cc. of 5 per cent guinea pig red cells. Incubation and ice box was for 2 hrs. at 38°C. and readings were made after the tubes had stood in the ice box over night.

Serum.		Serum dilution.								Complement + red cells + 0.2 cc. of salt solution.
		0	1/2	1/4	1/8	1/16	1/32	1/64	1/128	
Untreated immune.	A	C.	C.	C.	Alm. C.	Alm. C.	+++	Tr.	Ft. Tr.	0
	B	++++	+++	++	+	+−	Tr.	Ft. Tr.	0	
Untreated normal.	a	Tr.	Ft. Tr.	0	0	0	0			
	b	"	" "	Ft. Tr.	0	0	0			
Exhausted sera		No hemolysis by any.								

The selective absorption had completely deprived the immune sera of their relatively strong hemolysin.

Hemagglutination.—This was read in mixtures similar to the foregoing but containing chicken serum (1 in 10) as complement. None of the tubes showed any hemolysis with this complement, but those containing undiluted immune goose serum exhibited a slight hemagglutination. None of the exhausted sera agglutinated chicken cells in the least.

Precipitation.—The normal sera contained no precipitin, but a weak one was present in the immune sera. It was active against dilutions of chicken serum up to, and including, 1 in 40.

In Vivo Tests of Neutralization.—The exhausted sera only were used in neutralization tests. For this purpose mixtures were made of the sera with a Berkefeld filtrate containing the tumor-producing agent, and these after incubation were injected into fowls. In some early experiments mixtures of the filtrate with isotonic saline or Locke's solution were employed as controls, but it was found that they soon lost their tumor-producing activity when incubated, whereas this was retained in mixtures with normal goose serum, either untreated or exhausted. Consequently in the present experiment, as in others to be detailed, the mixtures with normal sera constitute the controls.

The tumor filtrate was prepared by grinding fresh neoplastic tissue with sand, making a thin suspension in Locke's solution, shaking, centrifuging, and passing the clear fluid through one or another of several Berkefeld filters (N). Several filters were used to ensure an active filtrate, since the tumor-producing agent is held back by many of the finer Berkefeld candles, and all the filtrates were united. Now two mixtures were made with the sera: (1) 15 cc. of each exhausted serum + 7 cc. of filtrate; (2) 7 cc. of each exhausted serum + 2 cc. of filtrate. These were incubated for 2 hours at 38°C. They remained water-clear. 1 cc. of a suspension of sterile diatomaceous earth was added to each, and portions of all were injected into each of a number of chickens. The mixtures with immune sera were injected first so that any possible advantage as regards attenuation of the virus during incubation, or neutralization of it, might lie with the mixtures containing the normal serum. Diatomaceous earth was added because, through the tissue injury it causes, the production of tumors by a filtrate is rendered much more certain.[6]

The ten chickens inoculated received 3 cc. of each mixture, into the pectoral muscles and the muscles of the upper wings respectively. Usually the tumor grows fastest and becomes largest in the pectoral muscles, and for this reason the injection site for the mixtures was varied from bird to bird; but in the experiment now under consideration no favoring influence of the pectoral situation

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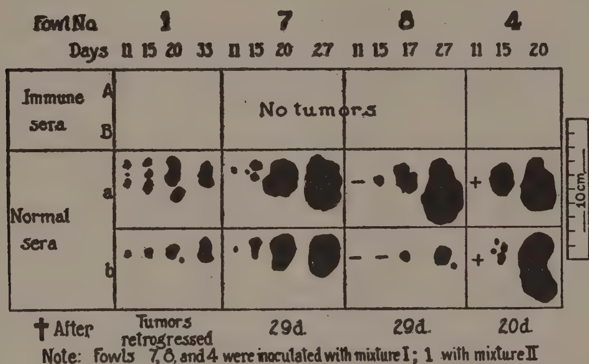
[6] Rous, P., Murphy, Jas. B., and Tytler, W. H., *J. Am. Med. Assn.*, 1912, lviii, 1751.

was to be seen. The growths did not attain a very large size before death ensued from metastases.

Clear-cut findings were obtained, as Text-fig. 1 shows. Only four of the ten fowls developed tumors. In them growths failed to appear where the mixtures of immune sera and filtrate had been injected, whereas at the control sites large ones developed.

Experiment 2.—The same general plan was followed as in the preceding experiment, but the immunized geese had now received two additional intraperitoneal injections. Bleeding for serum was done 121 and 103 days respectively from the time immunization of the birds was started, and 7 days after the last



TEXT-FIG. 1. The tumors in four fowls receiving intramuscular injections of mixtures of tumor filtrate with immune and normal goose sera respectively.

injection. The sera of three normal geese, a, b, and c, were used in control. Selective absorption was carried out as usual.

30 cc. of goose serum + 5.8 cc. of chicken red cells incubated 1 hr. and
serum transferred to 2.9 " " " " " " 1 " "
" " " 2.8 " " " " " " 1 "

Cultures taken after the last absorption proved sterile.

Anti-Chicken Titer of the Sera. Hemolysis.—0.2 cc. of inactivated serum in graded dilutions + 0.2 cc. of 1 in 10 guinea pig complement + 0.2 cc. of 5 per cent chicken red cells.

Serum.		Serum dilution.								
		0	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256
Untreated immune.	A......	C.	C.	C.	Alm. C.	++	+	Tr.	0(?)	0
	B......	Alm. C.	Alm. C.	+++	++	+	Ft. Tr.	0	0	0
Untreated normal.	a......	No hemolysis.								
	b......	+−	0	0	0	0				
	c......	+++	++	+	0					
Exhausted sera......		No hemolysis by any.								

Hemagglutination.—0.2 cc. of inactivated serum in graded dilutions + 0.2 cc. of 5 per cent chicken red cells + 0.2 cc. of salt solution.

Serum.		Serum dilution.				
		0	1/2	1/4	1/8	1/16
Untreated immune.	A..............	+	+	+	Tr.	0
	B..............	++	Tr.	0	0	0

With the exhausted normal and immune sera, as well as the untreated normal sera, no agglutination was obtained.

Precipitation.—There was no precipitin in the normal sera, but one was present in that from both immune geese. It was effective in mixtures of equal parts of the undiluted goose serum with dilutions of chicken serum up to and including 1 in 40 for Goose A and 1 in 20 for Goose B. The titer was little if at all diminished by the absorption with red cells.

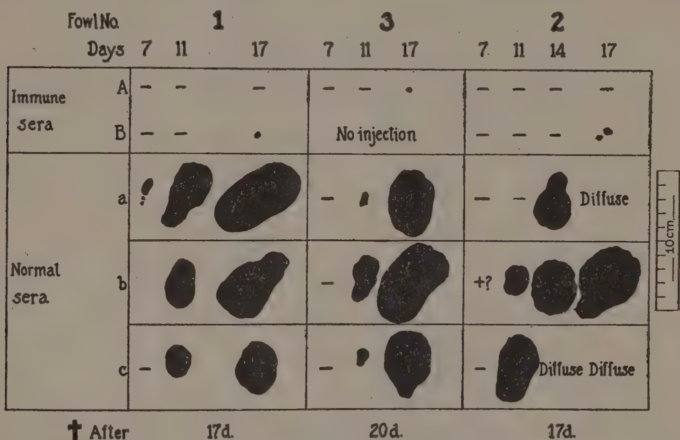
In Vivo Tests of Neutralization.—A Berkefeld filtrate of a tumor extract was prepared by the method already described, and three mixtures were made of it with the exhausted sera, both normal and immune.

Proportion X: 7.5 cc. of serum + 2 cc. of filtrate.
Proportion Y: 12 cc. of serum + 6 cc. of filtrate.
Proportion Z: 7 cc. of serum + 7 cc. of filtrate.

Incubation was for 2 hours at 37°C. No precipitation or clouding occurred. A suspension of diatomaceous earth was now added to each mixture in the amount of one-tenth its volume, and the injection of fowls was forthwith begun. Fifteen fowls were used, and all save four received 3 cc. of each mixture, the site of injection being varied. The four fowls mentioned were not given the mixture con-

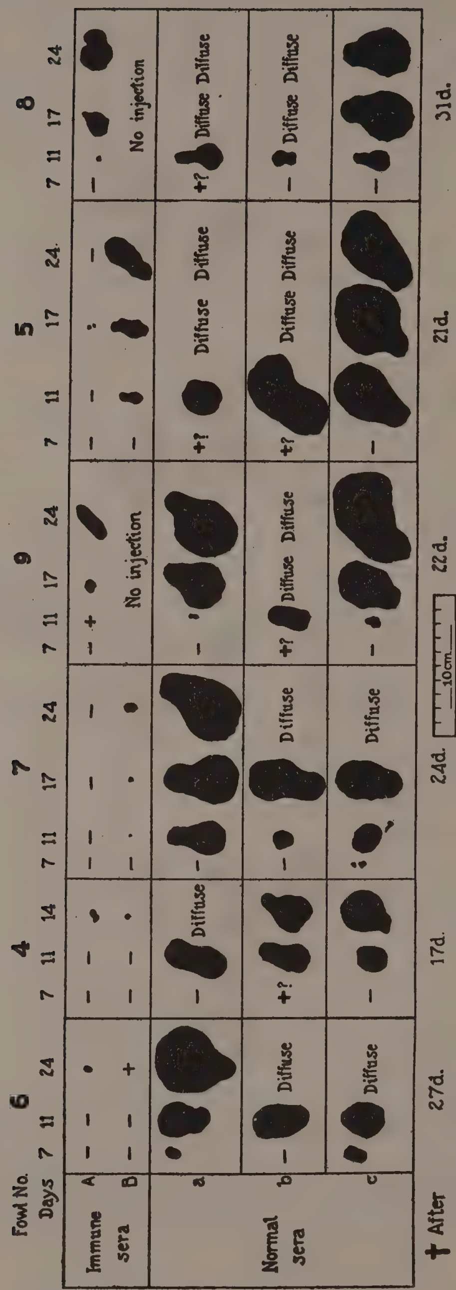
taining the serum of Immune Goose B. The injections were made into the upper wing, upper leg, and pectoral muscles. As Text-figs. 2, 3, and 4 show, large growths rapidly developed where the control mixtures had been placed, whereas none, or only slowly growing ones, were caused by the mixtures containing immune serum.

The neutralizing effect on the tumor-producing agent of the exhausted serum of geese immunized with tumor tissue is clearly shown by these protocols. The agent was especially active in the filtrate used in Experiment 2, as shown by the fact that every one of the fifteen inoculated fowls developed tumors—an occurrence unparalleled in our records. The immune serum completely prevented

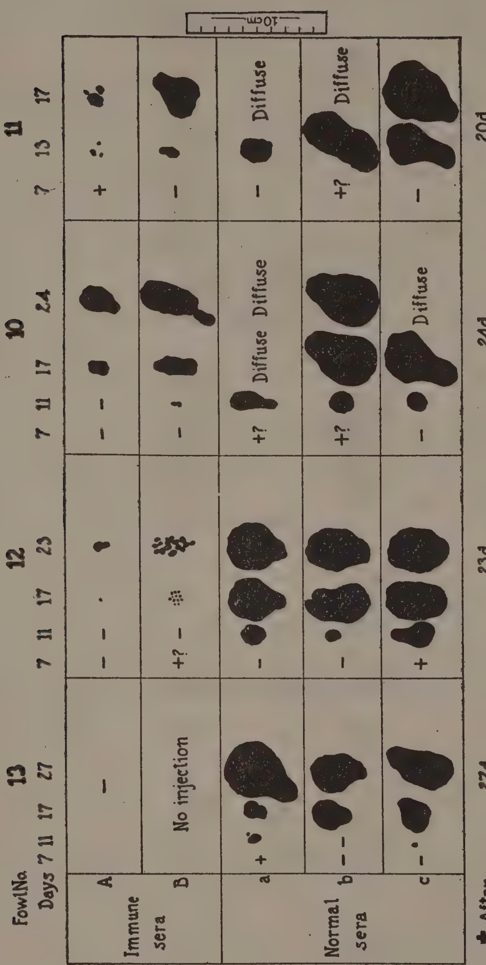


TEXT-FIG. 2. The tumors developing in three fowls receiving mixtures in Proportion X.

tumors at only three injection sites in these fowls, though its protective influence was manifest wherever it had been injected. Very large tumors resulted from all three normal serum mixtures, whence it may be inferred that even the smallest amount of filtrate present in any one, namely that of Proportion X (about 0.66 cc. of filtrate per fowl), contained what might be termed a maximum tumor-producing dose of causative agent. More than twice this amount (1.5 cc. in Proportion Z) yielded tumors that were no larger and grew no more rapidly. The test of the neutralizing power of the immune sera was evidently a severe one in this experiment. In Experiment 1 the filtrate was far less active, as shown by the large proportion of nega-



TEXT-FIG. 3. Tumors in six fowls receiving mixtures in Proportion Y.



TEXT-FIG. 4. Tumors in four fowls receiving mixtures in Proportion Z.

tive fowls (six out of the ten inoculated) and the slow course of the tumors that appeared. Here the neutralization of the tumor-producing agent by the exhausted serum of the immunized geese was complete.

To what is the neutralization referable,—unabsorbed remnants of chicken antibodies? This possibility may be tested by determining whether chicken antibodies as such are able to neutralize the tumor-producing agent. The results with the sera of immunized rabbits gain importance in this connection. For the rabbit sera, while strongly anti-chicken—many times more so than the goose sera—had not the least neutralizing effect on a tumor filtrate.

Experiment 3.—A rabbit was given three intravenous injections on successive days of a saline extract of chicken tumor, followed at 6 day intervals by eight intraperitoneal inoculations of a mixture of tumor suspension and citrated blood from fowls moribund of the growth. 8 days after the last injection the animal was bled to death, and its inactivated serum was compared in neutralizing power with that of a normal rabbit. Selective absorption of both was carried out as usual.

Mixture.	Hemagglutination.
15.5 cc. of rabbit serum + 4 cc. of chicken red blood cells, incubated 1 hr. and	Marked.
serum transferred to 4 cc. of chicken red blood cells, incubated 1 hr.......	0

Anti-Chicken Titer of the Sera. Hemolysis.—0.25 cc. of inactivated serum in graded dilutions + 0.25 cc. of 1 in 10 guinea pig complement + 0.25 cc. of chicken red cells.

Immune serum.	Serum dilution.															Guinea pig complement + salt solution + red cells.
	0	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1,024	1/2,048	1/4,096	1/8,192	1/16,384	
Untreated..	C.	C.	C.	C.	C.	C.(?)	Alm. C.	+++	+++	+++	+	±	Tr.	Tr.	Ft. Tr.	0
Exhausted..	+	+	±	±	Tr.	Tr.	Tr.	F. test.	0	0						

Exhaustion was in this instance only approximately complete.

Hemagglutination.—The mixtures were the same as those above except that 0.25 cc. of 0.9 per cent salt solution was substituted for guinea pig complement.

Immune serum.	Serum dilution.									
	0	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512
Untreated....	+++	Alm. C.	Alm. C.	C.	C.	++	++	Tr.	—	0
Exhausted....	No agglutination.									

The normal rabbit serum destined to be used in control of the *in vivo* work caused only the slightest hemolysis of chicken cells and no agglutination, when tested prior to its absorption. Thereafter it did not affect the cells at all.

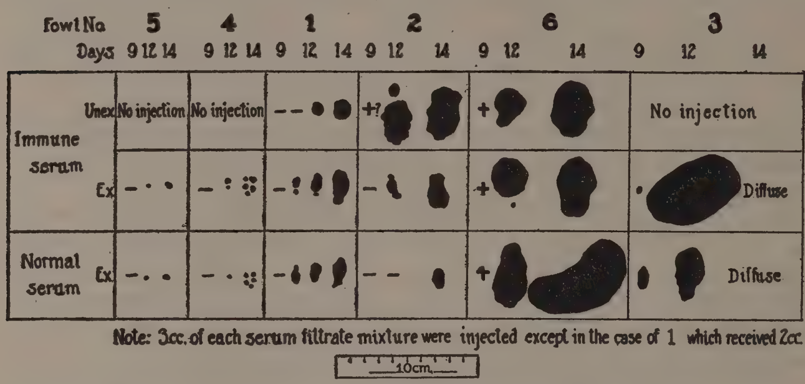
Precipitation.—The normal rabbit serum was entirely inactive, but that of the immunized animal caused precipitation when incubated with equal parts of chicken serum diluted up to and including 1 in 2,560.

In Vivo Tests of Neutralization.—Three serum specimens were used—normal and immune serum, exhausted as above, and untreated immune serum. A Berkefeld filtrate containing the tumor-producing agent was prepared as usual and mixed with the rabbit sera in the proportion of 6 cc. of filtrate to 12 cc. of serum. Incubation at 38°C. was carried on for 2 hours, cultures were taken, portions of a suspension of diatomaceous earth in salt solution were added to each mixture (0.7 cc. for every 20 cc. of mixture), and injections were made of 3 cc. into five fowls and of 2 cc. into a sixth. In the mixtures with immune serum a floccular precipitate had come down which was distributed by shaking prior to the injections. The sites of injection were varied, as usual. The cultures of the injection fluids were negative after 2 days. Tumors developed in all the fowls, as Text-fig. 5 shows.

The test of the neutralizing power of the rabbit sera was in this case not a severe one. For the late appearance and slow growth of the control tumors clearly showed that no excess of tumor-producing agent was present in the mixtures. Yet there is not the slightest indication of any effect upon the agent of the immune serum, even when it had not been exhausted and was very strong in chicken hemolysin, agglutinin, and precipitin. Said serum had exactly the same effect as serum from a normal rabbit, which contained only the weakest antibodies for the chicken. A floccular precipitation occurred in the mixtures of filtrate and immune serum, but so slowly that it can scarcely have afforded to the tumor-producing agent much protec-

tion from other serum antibodies; and only complete protection by it would explain the results in the inoculated fowls.

This experiment would seem to prove that the neutralization of the tumor-producing agent by the serum of immunized geese is not due to antibodies directed against chicken tissue as such. Such antibodies —or at least those elicited in the immunization of rabbits—fail entirely to injure the tumor-producing agent, even when they are very strong. In view of these facts, the conclusion seems justified that the neutralization of the agent causing a chicken tumor by the serum



Text-Fig. 5. Tumors arising in six fowls injected with tumor filtrate mixed with normal and immune rabbit serum.

of geese repeatedly injected with the tumor tissue is not the result of the action of antibodies directed against the chicken tissue as such, but is due to others specific for the tumor-producing agent. These are retained by goose serum exhausted with chicken red cells.

DISCUSSION.

The selective absorption of tissue antibodies has been applied thus far to four immune sera of widely different properties (see Part I of this paper), with success in each instance. There is no doubt that by the method sera can be deprived of antibodies immediately injurious to the animal organism while retaining those directed against an infectious agent or its products. Applications of the principle in the treatment of disease at once suggest themselves. But many points

must be determined before any practical therapeutic venture is warranted.

First, the late or latent effects on the animal body of exhausted serum must be closely studied. Serum precipitins are not removed with hemolysins and hemagglutinins during the process of exhaustion with red cells. What then is the effect of a specific precipitin acting *in vivo* on an animal of the species against which it is directed? We have been unable to find in the literature a conclusive answer to this obvious question. The controversy over the relation of precipitation to anaphylaxis has resulted in a multitude of *in vivo* experiments, but these have been carried out almost exclusively by introducing precipitin and precipitinogen into animals to which both are alien, or by injecting a serum precipitinogen into an organism that possesses, or will develop, a precipitin for it. Uhlenhuth and Haendel[7] and Doerr and Moldovan[8] have claimed that anti-guinea pig rabbit serum of high precipitin titer is toxic to guinea pigs when injected intravenously; but these authors made no attempt to absorb from the serum the hemolysins and agglutinins present in it and undoubtedly capable of harmful effects. Their work has not been followed up. We plan to do this.

It seems not unlikely that an antiserum resulting from injections of tissues, especially tissues other than blood, will contain elements of possible harm besides hemolysins, hemagglutinins, and precipitins. Here one is confronted with the problem of the specificity of cytotoxins, so long and indecisively debated. Fortunately we are concerned with a single aspect of this problem; namely, that of whether specific cytotoxins, assuming that they exist for the generality of organs—a large assumption—can be removed from serum by its exhaustion with red corpuscles. For should they not be so removable it may be necessary to exhaust a serum with the same kind of tissue employed in the immunization, a matter of much practical difficulty. Experiments on the point with a specific cytotoxic serum, so called, have been begun.

Theoretically the most important use of exhausted sera lies in the treatment of infections of unknown cause. And with each such in-

[7] Uhlenhuth and Haendel, *Z. Immunitätsforsch., Orig.*, 1910, iv, 761.

[8] Doerr, R., and Moldovan, J., *Z. Immunitätsforsch., Orig.*, 1910, vii, 223.

fection two fundamental points would have of necessity to be determined. They are (1) whether the infected tissue will suffice as a practical antigen, and (2) whether the antibodies useful against the infection or its products will survive the serum's exhaustion of antibodies injurious for tissue. The microorganisms in infected tissue employed as antigen will be in many instances in the highest state of pathogenicity. There are advantages to this, but also drawbacks. If the animals to be immunized are themselves susceptible to the infection much less fresh tissue antigen can be employed than of one attenuated by culture or in another way. The dosage of antigen will also be difficult to regulate. Both these obstacles were encountered in Part I of the present work, during our attempts to immunize dogs by injecting them with the blood of rabbits dying of pneumococcus septicemia. So large a percentage of the dogs died that resort was had at length to an antigen of normal tissues and pneumococcus cultures injected separately. The conditions would be much more favorable to successful immunization in the case of infections only slightly pathogenic to the animals employed for immunization. Here tissue containing the infective agent in most virulent form would have great advantages and not improbably decisive ones in the case of cultivable agents that lose their pathogenicity, and incidentally their usefulness as antigen, when grown *in vitro*. Furthermore, it is conceivable that with an agent in highly virulent form so little of the tissue containing it might in certain instances be required as antigen that the serum's titer in elements injurious for tissue would be slight, and the exhaustion in consequence a relatively simple matter.

Little can at this time be said on the persistence of desirable antibodies in an exhausted serum, further than that our experiments make this seem probable in most instances, as do also the observations of others who have used the method of selective absorption to a different end; namely, to demonstrate the specificity of antibodies.[9] Should it become necessary to exhaust a serum of precipitin by means of precipitation in order to render it harmless *in vivo*, even this, it

[9] A noteworthy demonstration of the possibilities of the method is to be found in the work of Todd, C., and White, R. G., *Proc. Roy. Soc. London, Series B*, 1910, lxxxii, 416. By the selective absorption of induced isohemolysins these authors were enabled to recognize the red corpuscles of individual oxen.

would seem, might be done without, in most instances, removing the antibodies directed against an infectious agent. For Gay and Stone[10] have made many attempts to bring down such elements in a serum precipitate, but without success.

Although the use of exhausted serum in the treatment of infectious diseases is at present but a distant possibility, there lies open a field for its immediate employment. Through the method of absorption much may be learnt regarding serum immunity to animal diseases—as witness the case of the chicken sarcoma,—and to human infections of unknown cause that are transmissible to animals. For the tissues of infected animals will furnish a ready antigen for experimental purposes, while normal individuals of the same species can be used as test objects to determine whether the exhausted sera resulting from immunization possess any protective power. A concrete illustration of such a possibility is afforded by some recent work of Nicolle and Blaizot.[11] These authors state that they have produced an effective antityphus serum in donkeys by injection with the spleens of guinea pigs dying of the disease. The serum is intended for use in human beings, but they find that with it guinea pigs can be cured of typhus, though the serum is so toxic for such animals that it can be given only in small quantities, which hinders the tests. It would have been interesting to deprive the serum of this toxicity by selective absorption with guinea pig cells, with a view to a more striking demonstration of its antityphus power.

SUMMARY.

By the method of selective absorption with tissue, protective serum antibodies have been demonstrated in the case of an infection of unknown cause; namely, a chicken sarcoma transmitted by a filterable agent. Geese were repeatedly injected with the finely ground sarcoma and with blood from fowls moribund of it; and their sera acquired the power to prevent the tumor-producing agent from causing growths. That this was not due to antibodies elicited by the chicken tissue as such was shown by exhaustion of the goose sera with chicken

[10] Gay, F. P., and Stone, R. L., *J. Immunol.*, 1916, i, 83.

[11] Nicolle, C., and Blaizot, L., *Ann. Inst. Pasteur*, 1916, xxx, 446.

red cells, a step which had not the least effect on the tumor-preventing power, and also by experiments with rabbits immunized as were the geese. These animals developed strong chicken antibodies in their sera which failed nevertheless to affect the tumor-producing agent.

Serum immunity to the chicken sarcoma is weak at best; and in the case of some other infections of unknown cause, more striking results may be anticipated from the method of selective absorption. It is even conceivable that by its means sera of therapeutic usefulness may become available. But much remains to be settled as regards the dangers of exhausted sera and the limitations of the method. Fortunately there exists an immediate field for the latter in laboratory studies on the nature of immunity to infections of which the cause has not been recognized.